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PATHOLOGY OF TOTAL BODY IRRADIATION IN THE MONKEY*

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The structural and functional changes occurring in rhesus monkeys following total body irradiation are being studied by a group of investigators at the Ohio State University College of Medicine. As a preliminary, it was necessary to determine the dose of radiation lethal to 50 per cent of the animals within 30 days of exposure (LD 50/30). The present paper is limited to a study of the 92 animals dying during that initial phase of the work (Table I).

To minimize extraneous factors that might affect survival of the animals, the monkeys were handled only at the time of irradiation. Unfortunately, this precluded carrying out any procedures such as weighing, blood counts, and bacterial cultures while the animals were alive. Under these conditions the LD 50/30 was approximately 550 r.; the LD 100/30 was 800 r. (Text-fig. 1).

MATERIALS AND METHODS

The experimental animals, rhesus macaques (*Macaca mulatta*), which are native to northern India, were purchased from an animal importer. The age of the animals was fixed at 1½ to 3 years based on roentgenograms of the jaws which revealed the presence of unerupted teeth.^{1,2} In the rhesus monkey puberty begins at about 3 years, but reproduction is not regular until the age of 4½ years.³ All animals were tuberculin tested, but owing to lack of space it was not always possible to isolate all new arrivals.

The cages measured 2 feet high by 2 feet square and were of heavy sheet metal; the front and top only were fenestrated. Two monkeys

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were kept in each cage; however, it was necessary to have cage mates of approximately equal size and temperament to avoid injury and malnutrition of the weaker.

The animal quarters were in an air-conditioned room kept at 72° F.; it measured 22 by 15 feet and had a very high ceiling. Large windows stretched across one entire wall, providing adequate daylight.

The monkeys were fed three times daily. At noon they received an apple or orange; in the morning and late afternoon they were fed a moist mash made by adding water to the dehydrated Okatie Farm

TABLE I
Irradiation Dosage and Survival Period

X-ray dosage	Total no. of animals	Animals surviving 0-30 days	Animals surviving 31+ days	Animals surviving 31+ days
r.				%
900	10	10	0	0
800	8	8	0	0
700	23	21	2	8.7
600	9	5	4	44.4
500	22	9	13	59.0
400	8	2	6	75.0
300	12	1	11	91.7

Primate Diet.* This ration is composed of ground wheat, soy bean meal, and dry powdered milk to which has been added alfalfa meal, sugar, bone meal, calcium carbonate, sodium chloride, viosterol, thiamine, niacin, folic acid, riboflavin, pyridoxine, and calcium pantothenate.

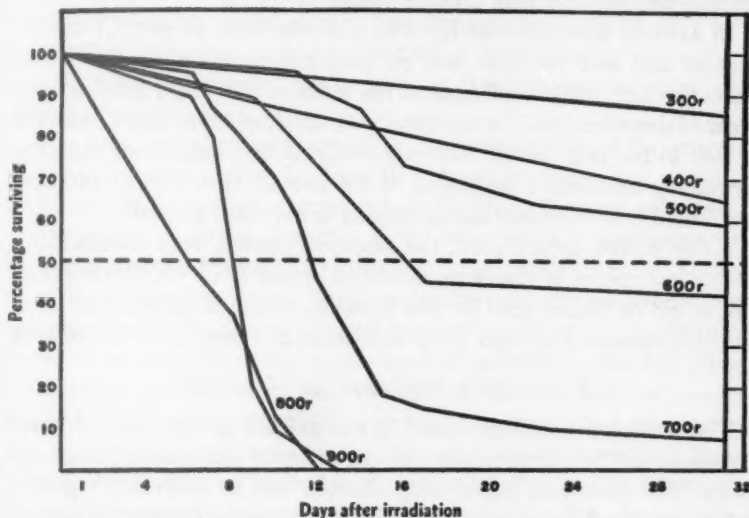
The techniques and methods employed in irradiating the monkeys were planned and supervised by Dr. Joseph Morton of the Department of Radiology. A 250 kv. Quadrocondex therapy machine was used at 15 ma. with a filter of 0.5 mm. copper and 1.0 mm. aluminum. Each animal was irradiated separately while being rotated at 1.3 r.p.m. 1 meter from the x-ray tube. The monkey was in an upright position within a cylinder 20 inches long having an inside diameter of 6 inches. Masonite plates were fitted into each end of the cylinder to prevent undue movement of the monkey during exposure. The exposure rate was 23 r./min.

The wall of the cylinder was of $\frac{5}{16}$ inch thick polyethylene containing carbon black and was perforated by holes $\frac{1}{2}$ inch in diameter. These were uniformly distributed as 2 holes per square inch of sur-

* Okatie Farms, Pritchardville, S.C.

face area. The density of the polyethylene was 0.93, but with 40 per cent of the wall replaced by holes, the effective density of the cylinder was 0.54. A Victoreen r. meter was used before and after each irradiation to measure the dosage being delivered. The meter was one of a paired set calibrated by the Bureau of Standards for use in this project. The dosage in air outside and inside the cylinder was the same within the limits of error imposed by the technique of measurement. This lack of differential may be due to absorption by the wall of the cylinder equaling scatter in the air inside the cylinder. No other object that might be a source of scatter was within one meter of the tube.

Necropsies usually were performed shortly after death of the ani-



Text-fig. 1. Mortality curves for the first 32 days following 300 to 900 r. total body irradiation of the monkey.

mal. However, in some instances when the monkey died during the night the time lapse amounted to several hours. Bacterial cultures were routinely taken of the heart's blood, liver, spleen, kidney, and ileac mucosa. Evisceration and inspection of all organs, including femoral marrow, brain, spinal cord, and eyes, were carried out. Before fixation of the specimens, significant lesions were photographed in color. Selected pieces of tissue from all organs were placed in Zenker-formol solution for immediate fixation and subsequent microscopic examination. All viscera, including the face and base of the skull with the middle ear, were placed in 10 per cent formalin for further study when needed.

POST-IRRADIATION SICKNESS

All monkeys subjected to 300 to 900 r. total body irradiation developed anorexia within 24 hours of exposure. In animals receiving low doses of radiation (300 to 400 r.) the loss of appetite was transient and unaccompanied by other signs of illness. More intense radiation (500 to 700 r.) was frequently followed by diarrhea which, in animals receiving 800 or 900 r., was severe and watery; however, rarely was any blood streaking noted. Vomiting was not observed, although thirst, probably largely due to the diarrhea, was a prominent feature. Eldred and Trowbridge⁴ noted vomiting in some of their monkeys, but this was seen in animals fed shortly before radiation. None of our animals was fed less than 4 hours before exposure.

In animals that survived for 2 to 3 weeks (500 to 700 r.) the fur became dull and the coat was no longer smooth. After the second week the hair rubbed off against the sides of the cage, leaving large areas of denuded skin. In monkeys that survived there was a regrowth of hair in the bare areas. Animals receiving this amount of radiation frequently manifested an edema of the muzzle area that at necropsy was found to accompany an underlying necrotizing gingivitis.

A day or two before death the monkeys became very listless, often sitting in a corner of the cage with head bowed (Fig. 1). Occasionally one would be found dead in this position, although more often it lay on the bottom of the cage. They died without evidence of convulsions.

PATHOLOGIC FINDINGS AT NECROPSY

The pathologic changes found in the animals at the time of death are presented by organ systems in the order of decreasing radiosensitivity. The gross and microscopic changes will be described together.

The averaged organ weights of 12 normal non-irradiated immature monkeys measured in grams were: heart, 11.0; right lung, 12.5; left lung, 11.0; spleen, 3.0; liver, 95.0; both adrenal glands, 2.0; each kidney, 8.5; and the brain, 82.0. These weights were likewise representative of those found in the irradiated animals except that the lungs were often heavier due to hemorrhage and edema. The spleen and adrenal glands also often varied from the normal (Table II).

Bone Marrow

The femoral marrow was always dark red; occasionally it appeared translucent, but never was it gray or fatty. This may have been due in part to the immaturity of the monkeys. No animal died less than 6 days after irradiation; those that died on that day had received 900, 800, and 700 r., respectively. In these there was an almost com-

plete absence of normal marrow elements; erythrocytes distended the sinuses and gave the marrow its dark red gross appearance. Small numbers of mature neutrophils and megakaryocytes were present, but immature forms of the erythrocytic and granulocytic series had wholly disappeared and were replaced by scattered reticulum cells (Fig. 2). In monkeys receiving 500 or 600 r. and dying within 14 days of exposure the findings were similar. In an animal that died 10 days after 400 r. irradiation, the marrow did not have the aplastic appearance found at higher doses. Although it was less cellular than normal, clumps of erythroblasts and normoblasts as well as an occasional myeloblast were seen; however, many of these cells showed nuclear abnormalities such as vacuolization and pyknosis. Only one of the monkeys receiving 300 r. died in less than 30 days. At 18 days after irradiation there was evidence of extensive destruction of marrow elements; but regeneration, particularly of the erythrocytic series, was already present.

Regeneration after large doses of x-irradiation could be observed only in the 700 r. series because all animals receiving 800 or 900 r. had died within 13 days of exposure; at this time there was no evidence of regeneration, although rather large collections of megakaryocytes that had escaped destruction were seen occasionally (Fig. 3). Seventeen days after irradiation with 700 r. the marrow contained many reticulum cells and islands of erythropoiesis; cells of the granulocytic series were rare. Transition stages between reticulum cells and plasma cells similar to those described by Liebow, Warren, and DeCoursey⁵ for man also were present. At 27 days post-irradiation the marrow was very cellular and presented the characteristic appearance of hyperplasia. Although the majority of the cells were erythroblasts and normoblasts, some myelocytes could now be recognized. In an animal dying 61 days after 500 r. x-irradiation the marrow showed evidence of erythropoiesis and myelopoiesis (Fig. 4); however, there was considerable variation in the rate of recovery between different individuals.

Spleen

In animals that died 6 days after exposure to 800 or 900 r. the splenic capsule was slate gray and finely wrinkled; on section the cut surface of the spleen was dark red, follicles and trabeculae could not be recognized. Histologically, the normal follicular architecture had disappeared; only isolated lymphocytes were still present (Fig. 5). Nearly all that remained were the fibrous trabeculae, reticulum cells, and dilated sinuses in which the endothelial nuclei were so close together they appeared like beads on a string. Foci of fibrinoid necrosis

TABLE II
Gross Findings at Necropsy

Monkey no. and dosage	Survival period (days)	Sex	Organ index = organ wt. (gm.) body wt. (gm.) × 1000		Oral mucosa	Skin		Stomach	Small intestine	Colon		Lungs			Remarks		
			Spleen Control = 1.3	Adrenal gland Control = 0.87	Hemorrhage	Necrosis	Hemorrhage	Necrosis	Hemorrhage	Ulceration	Hemorrhage	Hemorrhage	Ulceration	Hemorrhage		Edema	Consolidation
					Hemorrhage	Necrosis	Hemorrhage	Ulceration	Hemorrhage	Hemorrhage	Ulceration	Hemorrhage	Edema	Consolidation			
900 r.																	
22	6	F	0.77	0.85										+			
18	7	M	1.00	0.50							+						
60	8	F	0.78	0.97										+			
62	8	M	2.32	1.39							+	+					
69	8	M	0.99	0.59							+			+			
57	9	M	0.63	0.63						+				+			
2	9	M	0.70	0.70							+	+					
61	9	F	0.81	1.22							+			+			Hyperemia of pia and arachnoid
63	10	F	1.07	0.86							+						
20	13	M	0.77	0.77							+					+	
800 r.																	
19	6	F	0.55	0.77								+					
26	6	F	1.25	1.30							+			+			
27	6	F	1.04	1.37								+	+	+	+		Hemorrhage in left adrenal gland
28	6	F	1.63	1.21				+			+	+					Chronic pulmonary tuberculosis
70	8	M	1.28	0.95								+		+			
24	9	F	1.11	0.89								+	+				
5	10	M	0.61	0.51			+					+	+				Hemorrhage in both adrenal glands
64	12	F	0.77	0.61				+	+					+	+		Petechiae in liver
700 r.																	
84	6	F	2.21	0.89								+					Stomach dilated
21	7	F	1.51	0.75								+					
31	7	F	0.89	0.46									+				Hyperemia of pia and arachnoid
16	8	M	1.00	0.88					+			+					
33	9	M	0.94	0.75					+			+	+		+		
120	9	F	1.18	1.41								+	+				
10	10	M	0.87	0.93			+					+					
29	11	F	0.46	0.74	+	+			+		+	+	+	+	+		Blood in gastro-intestinal tract
73	11	F	1.55		+	+			+			+	+				Hemorrhage in bladder
32	12	F	1.23	0.69		+			+					+		+	Dilated common bile duct
105	12	F	1.46	0.67	+	+	+		+				+	+			Blood in left pleural space
79	12	M	1.97		+	+	+							+			Epicardial petechiae
56	12	M	1.67	1.43		+							+				
96	13	F	1.13		+				+			+	+		+		Sanguineous fluid in abdomen, epicardial petechiae
88	13	F	0.81	0.61	+	+	+		+		+	+		+			Hemorrhage in pleura, epicardium and gallbladder
93	15	M	1.52	0.78	+	+					+	+		+			Ulcer on tongue; epicardial petechiae
134	15	F	1.47	0.84		+	+										
126	15	F	1.03									+	+	+	+	+	Generalized tuberculosis
66	15	M			+	+	+										Epicardial petechiae
30	17	M	1.75	0.90		+	+				+						Necrosis of cheek
77	23	M	1.80		+	+		+									
97	57	F	1.48	1.48								+	+				Not eviscerated; generalized tuberculosis
58	332	F															
600 r.																	
12	9	M	0.62	0.83								+	+				
3	13	M	0.42	0.53				+									
34	13	F	1.54	0.79				+				+	+	+	+		Epicardial petechiae
17	17	F	0.75	0.88	+	+			+							+	Epicardial petechiae

TABLE II (continued)

Survival period (days)	Sex	Organ index = organ wt. (gm.) body wt. (gm.) × 1000		Oral mucosa		Skin		Stomach		Small intestine	Colon		Lungs			Remarks
		Spleen	Adrenal gland	Hemorrhage	Necrosis	Hemorrhage	Necrosis	Hemorrhage	Ulceration	Hemorrhage	Hemorrhage	Ulceration	Hemorrhage	Edema	Consolidation	
		Control = 1.3	Control = 0.87													
17	F	0.91	0.72	+	+	+	+				+					Epicardial petechiae
167	M	1.87	0.53										+			Emaciation
183	F	1.27	0.95													Emaciation
401	F															Not eviscerated; general tuberculosis
496	M	2.54	0.27													Sacrificed; fibrous pleuritis
11	F	1.21									+	+	+			Petechiae in urinary bladder
12	M		0.78			+					+	+				Hemorrhagic cystitis
14	F	1.13	0.94			+		+	+		+	+				Subpleural hemorrhage
16	F	0.57	1.15			+								+		
16	F	1.10	0.55	+								+				Edema of face
16	M	0.94	0.37					+					+			
19	M	1.45	0.99	+	+								+		+	Conjunctival hemorrhage
22	F	2.10		+	+						+	+	+		+	Edema of face
29	F	1.86	0.83		+							+				
61	F	1.19	1.19												+	
69	M	1.33	0.57									+				Fibrous pleuritis
71	M	2.01	0.81													Pulmonary tuberculosis
84	F	1.25											+			
84	M	1.33	0.63			+					+		+		+	Epicardial hemorrhage
180	M	0.97	0.92								+				+	
231	M	1.76	0.28													Sacrificed
260	M															Generalized tuberculosis
262	F															Not eviscerated; generalized tuberculosis
323	M	1.84	0.26													Sacrificed
325	F	2.92	0.50													Sacrificed
396	F	1.45	0.12													Sacrificed
499	F	0.97	0.22													Sacrificed
10	M	1.39	0.84								+	+	+			
21	F	1.60	0.24												+	
33	M	0.84	0.99	+	+											Gangrene of face
80	M	2.04	0.92													Emaciation
156	F	1.31	0.91													Pulmonary tuberculosis
196	F	1.93									+	+				
324	M	1.78	0.32													Sacrificed; fibrous pleuritis
386	M	1.25	0.14													Sacrificed
13	F	1.14	0.49										+			Ulceration of skin
37	F	1.33	0.96													
41	F	1.47	0.84								+	+				Petechiae in bladder
90	M															Generalized tuberculosis
109	M	1.62	0.97													Generalized tuberculosis
131	M	2.18	1.06													Generalized tuberculosis
159	F															Not eviscerated; generalized tuberculosis
190	M															Not eviscerated; generalized tuberculosis
240	M	1.14	0.28													Sacrificed
311	M	1.52	0.19													Sacrificed
316	M															Sacrificed; not eviscerated; generalized tuberculosis
330	F															Sacrificed; not eviscerated; generalized tuberculosis

occasionally occupied the site of a former germinal center as indicated by the position of an arteriole.

In all animals dying within 10 days of irradiation, even if only 400 r. had been administered, the same widespread destruction of lymphocytes was apparent. Erythrocytes often flooded the pulp and distended the sinusoids; in several instances a ring-shaped hemorrhage was present surrounding the area formerly occupied by the germinal center (Fig. 6). The spleen of a monkey dying 18 days after 300 r. showed changes similar to those obtained with higher doses, although scattered lymphocytes remained.

Recovery was slow, usually initiated by the appearance of plasma (plasmacytoid) cells and proliferation of reticulum cells. Even 61 days after 500 r., repopulation of the follicle areas was just beginning. Monocytes in the sinusoids often contained hemosiderin. Well defined follicles were not present 84 days post-irradiation, and after 180 days were just beginning to appear, although lymphocytes now outnumbered plasma cells. Many of the lymphocytes had large and irregular nuclei.

During the next 80 days recovery continued slowly, and in animals surviving over 300 days there was almost complete restitution (Fig. 7). Subsequently, this went on to frank follicular hyperplasia that could be recognized grossly (Fig. 8); the hyperplasia was still manifest in a monkey sacrificed 499 days after irradiation.

There was little correlation between the weight of the spleen and the histologic findings. In many instances the spleen weighed more in animals dying within 1 to 2 weeks of irradiation than it did in others that had survived for many months after exposure (Table II). In irradiated rats and mice the splenic weight usually falls during the first week but is restored close to normal by the end of the second week.⁶⁻⁸

Lymph Nodes

The changes in the lymph nodes paralleled those found in the spleen. The nodes were gray-brown and seldom measured more than 6 mm. in diameter. Most of those examined lay along the aorta or within the mesentery. Six days after 900 r. only isolated lymphocytes remained scattered throughout the cortex. The greatly dilated sinuses contained large numbers of monocytes; within the cytoplasm of the latter there were often several erythrocytes (Fig. 9). Fibrin and monocytes often nearly filled the peripheral sinus (Fig. 10). After 12 days the plasma cells, which had previously been isolated, appeared in large foci, particularly in the areas of the cortex formerly occupied by lymphoid follicles (Fig. 11). The nuclei of the plasma cells were often large and hyperchromatic (Fig. 12).

The first animals to die after receiving 400 and 300 r. died in 10 and 18 days, respectively. The changes in the lymph nodes were similar to those observed after higher doses except for the presence of a few scattered lymphocytes in the nodes of the animal exposed to 300 r.

Recovery apparently began with the appearance of plasma cells which, 17 days after 700 r., had diffusely infiltrated the cortex. The monocytes still occupied the sinusoids of the medulla and in many instances contained hemosiderin—the last trace of the erythrocytes phagocytized earlier. After 23 days the number of lymphocytes in the cortex had increased slightly; follicles were not recognizable.

Similar changes were seen in animals exposed to 500 r.; and because many of these survived for long periods, subsequent events within the lymph nodes will be followed in them. After 61 days the single outstanding cell in the nodes was still the monocyte, which nearly filled the sinuses of the medulla; in the cortex only scattered clumped lymphocytes and plasma cells were present. The variety of cells, in addition to the occurrence of large and bizarre nuclei within many of the cells, gave the histologic sections a very pleomorphic appearance, which persisted for at least 180 days after irradiation. In the next 80 days the plasma cells were greatly reduced in number and the cortex became densely packed with lymphocytes. Monocytes were still prominent in the medullary sinuses. In animals surviving over 300 days the lymph nodes often reached a diameter of 1.0 cm. and had a nodular surface (Fig. 13) which, on section, was found to reflect the presence of hyperplastic follicles with large germinal centers (Fig. 14).

Other Lymphoid Tissue

The lymphoid tissue throughout the gastro-intestinal tract underwent changes similar to those observed in the lymph nodes. The solitary follicles of the colon were occasionally the seat of hemorrhage and necrosis. During the post-recovery period foci of hyperplasia also were seen (Fig. 15). The lymph follicles and aggregates in the nasopharynx were similarly affected. The thymus in the normal monkey of this age is beginning to undergo involution, but can usually be found quite readily. After irradiation, thymic tissue was identified with difficulty.

Gastro-intestinal Tract

The *esophagus* was the most radio-resistant portion of the gastro-intestinal tract. This may be due in part to the presence of squamous rather than glandular epithelium on the surface and the absence of lymphoid tissue in the submucosa. The esophagus was often hyperemic, but only occasionally was it the site of petechiae or shallow erosions.

The *gastric mucosa* frequently bore multiple, often confluent petechiae (Fig. 16); this was particularly apparent during the second week following irradiation with 700 r. In 2 of these animals there was extensive submucosal hemorrhage 8 to 12 days, respectively, after exposure. Gastric ulceration was observed only twice; in one monkey it occurred 12 days after irradiation with 800 r. and in another 14 days after exposure to 500 r. In the latter animal there was also extensive hemorrhagic gastritis; the rugae were greatly thickened and the mucosal surface covered by gray exudate.

Microscopically, the gastric mucosa 6 to 8 days after 800 or 900 r. still contained many plasma cells, as well as very occasional lymphocytes. The lymphoid tissue normally present in the submucosa had disappeared. The tubular gastric glands showed very slight distortion and no differential effect of radiation on the mucous, pepsin, or acid-secreting cells could be detected. The gastric ulcers observed in one animal 12 days after 800 r. were superficial, the deepest portions of the glands being intact. The "gastritis" observed grossly in another monkey 14 days after 500 r. likewise proved to be due to extensive superficial necrosis of the gastric mucosa. The fibrinous exudate contained necrotic epithelium and bacteria but no inflammatory cells.

The *small intestine* was singularly free of lesions when examined at necropsy. No ulcers were encountered, and in only 6 cases were petechiae seen in the mucosa, all occurring in the second or third week after exposure to 600 to 900 r. (Fig. 17).

The *colon* was more often the seat of hemorrhage or ulceration than any other part of the gastro-intestinal tract. The cecum and proximal portion of the colon appeared to be most vulnerable (Fig. 18), although the entire colon was usually affected in some measure. The incidence of petechiae and ulceration largely coincided, but in the lower dose range (300 to 500 r.) petechiae were more common. In all but 2 of the 25 cases showing ulceration of the colon, the animals died 6 to 13 days after irradiation; in the remaining 2, death occurred 17 and 22 days after exposure. The relation of colonic ulceration to amount of irradiation becomes apparent when the percentages of animals showing the lesion at necropsy are listed with the doses received: 900 r., 70%; 800 r., 50%; 700 r., 35%; 600 r., 22%; 500 r., 14%; 400 r., 12%; 300 r., 0%. In addition to petechiae, larger hemorrhages were frequent; they were often multiple, within the submucosa, and raised the mucosa to form a deep, bluish red, berry-like elevation approximately 1 cm. in diameter. Blood was rarely found free in the lumen.

The ulcers were multiple and ranged from 1 mm. to 2 cm. in diameter. Ulceration often appeared to begin with necrosis of a solitary lymphoid follicle; the immediately adjacent mucosa was hemorrhagic. In other cases the ulceration remained very superficial; the deeper portions of the tubular glands were intact and mitotic figures were often seen (Fig. 19). A few cells had large, irregular nuclei and vacuolated cytoplasm. The mucosa adjacent to such ulcers was intact except for loss of lymphocytes from the stroma; plasma cells and occasional eosinophils persisted. The lymphoid tissue normally found in the submucosa disappeared within a week of irradiation. Occasionally, weeks or even months after exposure, the glands were found to be dilated and the epithelial cells greatly flattened, leading to a pseudo-metaplasia with cells resembling squamous epithelium (Fig. 20).

Thrombosis of veins and occasionally of arterioles often was found in regions of ulceration. The rôle of infarction in producing the ulcers is difficult to evaluate; however, large vessels were never affected and in most cases thrombosis was quite clearly the result of ulceration and not its cause.

Mouth and Oral Pharynx

One of the striking post-irradiation lesions found in the monkey was necrotizing ulceration of the *gingiva* which closely resembled that observed in man.⁵ It was seen in 10 animals exposed to 700 r., in 2 after 600 r., in one after 500 r., and in one after 400 r. (Table II). The survival period of these animals ranged from 11 to 33 days, but 9 of the 10 receiving 700 r. died with necrotizing gingivitis 11 to 15 days after exposure. The presence of a severe gingivitis often was first manifested in the living animal by excessive salivation and edema of the muzzle. The edema frequently extended to involve most of the face, including the eyelids (Fig. 21). Occasionally a noma-like gangrene of the cheek was apparent (Fig. 22), and in one instance thrombosis of both external maxillary arteries produced a dry gangrene of the face (Fig. 23). Living animals were not examined, but on the basis of necropsy findings it appeared that the lesion began as hemorrhagic ulceration of the free gingival margin about a molar tooth, more often in the maxilla than in the mandible. The buccal or labial mucosa was secondarily involved in most instances; in 3 cases hemorrhagic necrosis was most marked in the lips.

Histologically, the early lesions were sharply limited shallow ulcers with a necrotic base in which bacterial colonization often was apparent. In the later stages with involvement of the buccal tissues, there was widespread, poorly delineated, hemorrhagic necrosis often asso-

ciated with tremendous edema of the adjacent tissues (Fig. 24). Inflammatory cells were absent from the affected areas in animals dying within 10 to 20 days of irradiation.

Although petechiae were frequently found in the *oral pharynx*, frank ulceration was encountered only once. The lesion occupied the region of the left tonsil and measured 1 cm. in diameter. The lymphoid tissue of the pharynx, however, uniformly suffered the changes seen in the abdominal lymph nodes.

Dr. D. W. Newman, working in this laboratory, made a careful study of the *salivary glands* and *teeth* from a representative series of the irradiated monkeys.² He examined the sublingual, submaxillary, and parotid salivary glands, as well as the buccal and labial accessory salivary glands. Although foci of parenchymatous degeneration were found, there were no changes that could be attributed directly to the effects of irradiation. Burstone,⁹ using P³² in mice and staining the glands with periodic acid-Schiff reagent, found evidence that, despite the absence of morphologic change, the cells elaborated a glycoprotein which was in a lower state of polymerization than normal.

Hemorrhage into the marrow spaces of the mandible occurred in varying degrees in the 16 animals examined. No hemorrhage was found in the pulps of developing teeth nor in the capsules surrounding them. There was no evidence of hypoplasia of enamel or dentin in the developing teeth such as was observed in young swine following 2000 kv. total body x-irradiation by English and Tullis.¹⁰ No changes were detected in the pulps of the erupted teeth, the periodontal membranes, or the alveolar bone.

The *lips* were rarely the primary site of oral ulceration. Although the labial mucosa was frequently involved by extension of the necrotizing ulceration of the gingiva, only twice were the lips alone affected. Both animals had received 500 r.; in one, dying 19 days after exposure, there was an encrusted ulcer in the skin of the upper lip as well as a hemorrhagic erosion on the mucosal aspect. The second animal died 29 days after irradiation and bore a shallow hemorrhagic ulcer on the mucosal surface of the lower lip.

The *tongue* was only twice the seat of post-irradiation ulceration and necrosis. One animal died 15 days after exposure to 700 r., the other 22 days after 500 r. In each case the ulcer was on the lateral border of the tongue (Fig. 25) and was continuous with hemorrhagic necrosis involving the floor of the mouth and gingiva. The epithelium adjacent to the ulcer showed very pronounced vacuolization and intercellular edema. Large colonies of bacteria were present in the ulcer base, but inflammatory cells were absent.

Skin

Changes in the skin of irradiated monkeys, though occasionally quite striking, were seldom contributory to death. Petechiae and epilation were directly due to x-irradiation. Petechiae first appeared 10 days after exposure to 500 to 800 r. and were rarely seen in monkeys that died more than 16 days following irradiation (Table II). Minute hemorrhages were scattered over the entire body, but were most prominent in the inguinal and axillary regions and over the abdomen (Fig. 26). The petechiae occasionally became confluent, but massive hemorrhage was observed in only one animal. This monkey died 13 days after receiving a dose of 600 r. and at necropsy a large ecchymosis was present on the anterior tibial aspect of one leg. This, however, was probably the result of trauma and was associated with hemorrhage into the adjacent fascia and muscle. The extensive edema and necrosis of the skin and subcutaneous tissue of the face observed in some animals were secondary to gingival necrosis and infection.

Epilation was not prominent until 12 days after irradiation, when it became apparent in many animals receiving 500 to 900 r. Thereafter, for a period of 2 to 3 weeks, the hair pulled out very easily at necropsy. In several instances large bare spots were present on the scalp, the side of the trunk, and the flanks where the monkeys had rubbed against the cage (Fig. 27).

Histologically, the changes in the skin were not very remarkable. The epithelium of the normal skin of the trunk is usually only 2 or 3 cells in thickness and devoid of rete pegs. After irradiation, particularly in animals receiving 600 to 900 r., the epithelial cells often showed extensive vacuolization in the cytoplasm with some distortion of the nucleus (Fig. 28). The connective tissue fibers of the corium were often coarse and dense, but whether this change can be attributed to irradiation is uncertain. Thrombi were not observed in the superficial vessels, although small hemorrhages were seen in the corium. Slight hyperkeratosis and plugging of the hair canals were found occasionally. At the base of the hair shaft the follicle often showed loss and distortion of the undifferentiated cells, accompanied by degeneration of the trichohyalin-bearing cells of Henle's and Huxley's layers (Fig. 29).

Respiratory Tract

The trachea was not affected severely by x-irradiation. The pseudostratified ciliated columnar epithelium was intact and without evidence of ulceration or metaplasia. Occasional nuclear abnormalities were found but their significance is doubtful. The mucous glands in the submucosa were frequently dilated and contained cellular debris and mucus.

The *lungs* were often the seat of hemorrhage and edema, particularly during the second week following irradiation with 700 and 800 r. (Table II). Diffuse hemorrhages, giving the cut surface a firm, somewhat dry, and dark red appearance, were common. In some instances these lesions closely resembled infarcts, but thrombosis of vessels could not be demonstrated. Focal areas of induration that were yellow and grossly resembled abscesses were seen occasionally, but microscopic examination showed that inflammatory cells were absent.

Histologically, the hemorrhagic regions were found to be made up of areas in which the alveoli were packed with erythrocytes, alternating with others that contained only edema fluid (Fig. 30). In these alveoli moderate numbers of macrophages were present; however, neutrophils and lymphocytes were absent. Occasionally the fluid was almost replaced by dense fibrin strands, resembling the condition found in lobar pneumonia, but minus the neutrophils. The presence of fibrin in the alveoli would suggest that the capillary endothelium had undergone great change in permeability. However, this may be attributed to the associated infection rather than to direct irradiation effect. In some instances the bronchiolar epithelium was destroyed as well as some of the mucous glands (Fig. 31). Large colonies of bacteria were present among the debris of the necrotic epithelium. Although normal lymphoid tissue was absent, groups of plasma cells often were present in the walls of the affected bronchi. Similar collections of plasma cells were seen also in normal bronchi. In animals that survived 6 weeks or longer, neutrophils were prominent in these lesions which then were comparable to the necrotizing bronchitis and bronchiolitis seen in unirradiated animals.

The *pleura* showed no characteristic lesions. In 2 monkeys that received 700 r. and died 12 and 13 days after exposure, sero-sanguineous fluid was present in the left and in both pleural spaces, respectively. In an animal which died 14 days after irradiation with 500 r. there were extensive subpleural hemorrhages but the pleura itself was intact. Many delicate pleural adhesions were encountered in 3 monkeys that survived over 60 days.

The Gonads

The sensitivity of the germinal epithelium to irradiation is well known. In the *testes* of these immature monkeys the usual stages of sperm maturation were absent and the effect of irradiation was less striking than in sexually mature animals. In all males examined the testes were still in the inguinal canal, where they normally remain up to and during puberty.¹¹ Grossly, the testes appeared normal. Histologically, the seminiferous tubules had very minute lumina lined by one

or two layers of primitive, undifferentiated germ cells. Scattered among these were very few spermatogonia having an eosinophilic cytoplasm and nuclei that frequently showed irregularity in outline and clumping of the chromatin (Fig. 32). The partial loss of spermatogonia and the evidence of damage to the nuclei of many that persisted were the only changes that could be related definitely to the effect of irradiation. They were produced by dosages ranging from 400 to 900 r. Similar changes have been observed in the testes of prepubertal rats subjected to 500 r. total body irradiation.¹²

The ovaries, though also from immature animals, showed the effects of irradiation more clearly than did the testes. Grossly, the ovaries were not abnormal, but microscopically there was evidence of extensive injury to ova. Most ova were surrounded by only one or two layers of follicle cells. In many instances the latter appeared unchanged; however, the ova often were shrunken, the cytoplasm dense and polychromatic (Fig. 33). The nuclei of the damaged ova exhibited clumping of the chromatin, pyknosis, karyorrhexis, or karyolysis. The ovaries of one monkey that died 10 days after exposure to 500 r. showed a remarkable change in the follicles. Many were large and contained 4 to 8 ova (or blastomeres?). Some of the large germinal cells were in intimate contact; others were partly separated by a few follicle cells (Fig. 34). The stroma of the ovaries showed no consistent change.

The vagina, uterus, and uterine tubes occasionally bore small submucosal hemorrhages; however, the epithelium remained intact.

Cardiovascular System

Except for frequent hemorrhages in the capillary bed, changes in the heart and blood vessels were not demonstrable. Thrombosis of larger vessels, e.g., in the muzzle area, and of arterioles and venules in the gastro-intestinal tract, probably were due to secondary infection rather than to direct radiation effect.

Capillary hemorrhages were most common during the second and third weeks after irradiation with 400 to 900 r. (Table II). At this time the platelets in the peripheral blood had begun to drop sharply¹³; the resultant thrombocytopenia was probably a prime factor in the hemorrhagic tendency. Tests for the presence of hyperheparinemia were not carried out at this time. Epicardial petechiae occurred in 8 animals (Fig. 35). In 7 monkeys they were found 12 to 17 days after exposure to 600 or 700 r., the remaining animal having survived 84 days after irradiation with 500 r. (Table II). Endocardial hemorrhages were seldom seen, even when epicardial petechiae were present. No

other gross or microscopic changes were found in the heart except for rare pseudocysts of sarcosporidia. These protozoan parasites stained normally in histologic sections and appeared unaffected by the irradiation.

Endocrine Glands

The *thyroid* and *parathyroid* glands were without gross or microscopic changes. The thyroid follicles were well filled with colloid that contained few vacuoles; the epithelium was low columnar and without papillary proliferation. The *pituitary body* appeared normal grossly. Histologic sections were characterized by good differential staining of the three major cell types and the absence of identifiable changes.

The *adrenal* medulla was free from hemorrhage or cytologic abnormalities. Occasionally, the cortex bore a few petechiae; twice there was a fairly extensive hemorrhage, both animals having been irradiated with 800 r. and having died 6 and 10 days, respectively, after exposure (Table II). In animals that died within 2 to 3 weeks of irradiation no consistent weight changes could be detected. However, with increasing time lapse between exposure and death there appeared to be a definite decrease in weight of the adrenal gland, particularly in animals that were sacrificed and were without chronic infection, e.g., tuberculosis (Table II). On the cut surface of all adrenal glands the cortex appeared gray rather than yellow, indicating loss of lipids. In histologic sections the cortical cells appeared less vacuolated than normal. This evidence of lipid depletion was present in animals that died several days or several months after exposure. The decreased size of the adrenal gland in monkeys that had survived for weeks or months after 400 to 700 r. was reflected in the narrow cortex. All three layers of the cortex were affected, but the atrophic change appeared most pronounced in the zona glomerulosa (Fig. 36).

Urinary Tract

The *kidneys* showed no gross evidence of radiation injury. In animals dying within 2 to 3 weeks of exposure to 700 to 900 r. there were histologic changes resembling so-called cloudy swelling or parenchymatous degeneration. Within the glomerular capsule there were often granular debris and occasional erythrocytes. The tubular epithelium showed slightly increased granularity of the cytoplasm; the lumina contained occasional hyaline and granular casts.

The *bladder* mucosa was the site of petechiae, particularly during the second and third week after irradiation. Rarely, small ulcers were present, but a hemorrhagic cystitis was not encountered.

Liver and Gallbladder

The *liver* was only once the site of grossly visible changes; *viz.*, numerous small hemorrhages were seen beneath the capsule and throughout the liver parenchyma in a monkey that died 12 days after 800 r. irradiation. In some instances there was slight edema of the peripheral connective tissue, but cellular changes were not striking. Fatty dystrophy, such as might occur in chronic tissue anoxia, was not encountered.

The *gallbladder* was found distended with blood in an animal that died 13 days after exposure to 700 r. No other lesions of the gallbladder were seen. Except for one case of a dilated common bile duct in which no calculi or other evidences of obstruction were found, the irradiated animals showed no abnormalities of the biliary system.

Pancreas

Both the exocrine and endocrine glands of the pancreas failed to exhibit any evidence of injury following irradiation.

Muscles, Bones and Joints

The *skeletal muscles* were occasionally the site of hemorrhage 2 to 3 weeks after irradiation. These could always be attributed to a previous trauma. Histologically, the skeletal muscle appeared normal. The bones and joints were likewise normal except in a monkey that died 332 days after exposure to 700 r. This animal had florid disseminated tuberculosis with tuberculous tenosynovitis and osteomyelitis in both feet. No direct effect of irradiation on bones or joints could be detected in any of the animals.

Nervous System

The *peripheral nerves* gave no evidence of radiation injury. The sympathetic ganglia between the muscular layers of the intestinal tract showed occasional cytoplasmic vacuoles and nuclear degeneration; however, this was very rare and probably was not a radiation effect.

The leptomeninges of the *brain* and *spinal cord* were often hyperemic, but petechiae were uncommon. Hemorrhage into the brain and cord was not observed nor were any other gross changes encountered. A detailed histologic examination has not been completed, but a preliminary survey shows no consistent alterations. Electro-encephalographic studies on monkeys receiving comparable doses of total body irradiation showed no significant changes.¹⁴ Direct irradiation of the brains of monkeys failed to produce recognizable cellular damage until

several thousand roentgens had been administered.^{15,16} However, Hicks and Montgomery¹⁷ have described necrosis of small subependymal cells of the lateral ventricles in young rats within 6 to 12 hours after as little as 200 r. total body irradiation.

The eyes were normal grossly; one animal that died 19 days after exposure to 500 r. bore a small conjunctival hemorrhage. A histologic study of the eyes of irradiated animals is in progress.

DISCUSSION

The functional and structural changes in the monkey following total body x-irradiation are strikingly similar to those described in man.^{5,18} The LD 50/30 of approximately 550 r. found in this study is midway between the figure of 500 r. given by Dowdy¹⁹ and the 600 r. of Eldred and Trowbridge.⁴ The sensitivity of the monkey to irradiation is less than that of the guinea-pig, swine, dog, and goat; greater than that of the rat, hamster, and rabbit; and about the same as that of mouse and man.²⁰ Because of the known influence of age on radiosensitivity,²¹ it must be emphasized that these were pubertal and prepubertal monkeys.

A very marked destruction of lymphocytes in the spleen and lymph nodes, as well as an aplastic appearance of the marrow, was found 6 days after irradiation. That these changes occur much earlier is shown by the fall of neutrophils and lymphocytes in the peripheral blood of monkeys within 6 hours of exposure¹³ and by the destruction of lymphocytes in the lymph nodes of swine 50 minutes after irradiation with 600 r. at 2000 kv.²² Destruction of blood-forming elements of the monkey by radiation has also been produced by the intravenous injection of radiostrontium²³; all animals died with a severe anemia.

The persistence and increase of plasma cells in the lymphoid tissue and bone marrow following body irradiation has been described in man,⁶ mouse,²⁴ and dog.²⁵ These cells were also prominent in the hematopoietic tissue of monkeys after exposure to x-rays; their origin from the radioresistant reticulum cells has been suggested.⁵ This view receives some support by the finding of intermediate forms between these two cell types in collections of plasma (plasmacytoid) cells in the irradiated monkey. Their functional significance remains obscure.

Flooding of the splenic pulp with erythrocytes, as well as erythrophagocytosis by the reticulum cells of the lymph nodes, may account for some loss of red blood corpuscles.²⁶ However, this bleeding into the lymphatics does not appear to be a major factor in erythrocyte destruction in the monkey.

One interesting finding was the occurrence of pronounced follicular

hyperplasia in the spleen and lymph nodes of some monkeys surviving over 300 days after irradiation. The well known lymphoma-producing effect of irradiation in mice²⁷ stimulated a careful search for any changes identifiable as lymphosarcoma or leukemia, but none were found. The single case of monocytic leukemia occurring in the human material studied by Liebow *et al.*⁵ was considered by them to be fortuitous. Nevertheless, the changes in the monkey, which in some measure are suggestive of giant follicular lymphoblastoma, warrant a careful follow up of animals surviving total body irradiation. The possibility of the late occurrence of lymphomas cannot be dismissed entirely.

The lesions found in the gastro-intestinal tract resembled in severity those of the swine, dog, and guinea-pig rather than of the rabbit and rat, in which the intestine is less affected.²⁸ The colon was often the seat of hemorrhage and ulceration, particularly during the second and third weeks after irradiation. Organisms found in blood taken from monkeys dying at this time were shown by Dr. Samuel Saslaw to be predominantly of the types normally found in the intestinal flora, but that are potential pathogens, *e.g.*, streptococcus, staphylococcus, and *Escherichia coli*.

In view of the reported frequency of petechiae and superficial ulceration in the small intestine of the mouse, the virtual absence of these lesions in the monkey is noteworthy. However, the small bowel injury in the mouse appears within the first 2 days after irradiation and then promptly heals.^{24,29,30} No monkey died before 6 days following exposure. This time lapse may well account for the absence of demonstrable lesions in the small intestine of these animals.

One of the outstanding post-irradiation lesions of man and monkey is a necrotizing gingivitis. Although the occurrence of an oral pharyngitis has been observed in other animals, particularly in swine,²² a gingivitis comparable to that found in man⁵ has seldom been reported. As was described, these lesions in the monkey usually begin at the free margin of the gingiva about the molar teeth; the gingiva in this area is particularly prone to trauma. This, coupled with the finding by Dr. Saslaw that streptococci were present in throat cultures of 80 per cent of the monkeys irradiated, may account for the high incidence of severe gingivitis in these animals.

The rôle of the adrenal gland in radiation sickness has been studied in man³¹ as well as in rats.^{5,32} Among the latter there was a significant increase of weight of the adrenal gland, occasionally up to 100 per cent, in animals that died within a few days after 650 to 900 r. irradiation.⁵ Such a sharp increase over the normal weight was not found in

the monkeys. Nevertheless, the weights of the adrenal glands in animals dying 1 to 3 weeks after irradiation were often higher than normal, whereas in monkeys dying 2 or more months later the adrenal glands were distinctly atrophic. This was associated with a loss of lipids and a narrowing of all layers of the cortex. Atrophic changes were most marked in the zona glomerulosa, a condition which has been described also in man.⁵

The mechanism of death within 1 to 3 weeks after irradiation is a complex of electrolyte imbalance associated with anorexia, diarrhea, and hemorrhage, of endocrine imbalance centering about disturbed adrenal cortical function, of lowered resistance to bacterial invasion due to impaired cellular and humoral defense mechanisms, and of increased exposure to infection as a result of injury to the epithelial barrier in the mouth and colon. Death that occurred many weeks or months after irradiation could usually be attributed to a generalized tuberculosis. This disease may have been present in a latent form at the time of irradiation, to become disseminated during the period of lowered resistance.

SUMMARY

To determine the LD 50/30 dose of total body x-irradiation for the rhesus monkey, 92 animals were exposed to doses ranging from 300 r. to 900 r. The LD 50/30 was found to be approximately 550 r.

The gross and microscopic lesions were strikingly similar to those seen in man. The hematopoietic system was affected first, with destruction of the blood-forming elements in the marrow and loss of lymphocytes from the lymph nodes and spleen. In animals that survived and were sacrificed several months after irradiation there was often a marked follicular hyperplasia in the spleen and lymph nodes.

Hemorrhage occurred most often as petechiae in the skin, lungs, epicardium, stomach, and colon. Epilation was noted about 12 days after irradiation.

Degenerative changes in the germinal epithelium of the ovaries and testes were observed following doses of 500 to 900 r. Atrophy of the adrenal cortex appeared as a late sequel to irradiation.

The colon was the site of hemorrhage and ulceration; however, within a week after irradiation with 800 r., active epithelial proliferation was seen in areas adjacent to foci of ulceration and necrosis.

Necrotic gingivitis and oropharyngitis was found in 14 monkeys receiving 400 to 700 r. and surviving 2 to 3 weeks. It closely resembled that observed in human casualties and began in the region of the molar teeth as a shallow hemorrhagic ulceration. Involvement of the buccal mucosa followed and was accompanied by edema which occasionally

involved the entire face. A noma-like necrosis of the cheeks may be the end result. Large ulcers along the lateral border of the tongue were observed in some cases.

The kidneys, liver, pancreas, central and peripheral nervous systems, muscles, bones, and joints were without changes directly attributable to irradiation.

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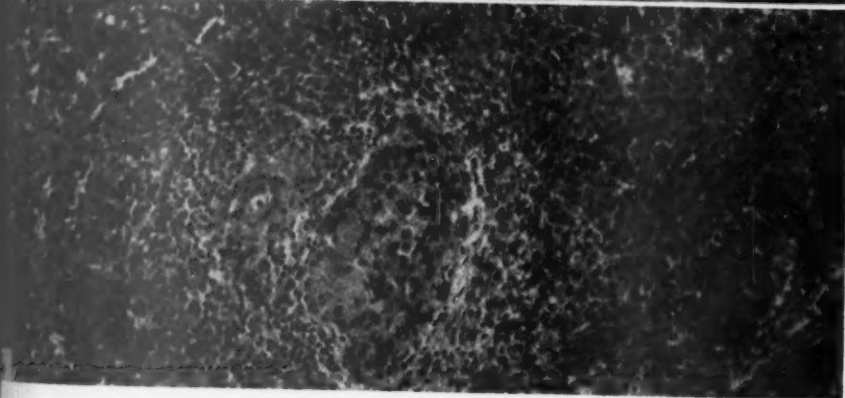
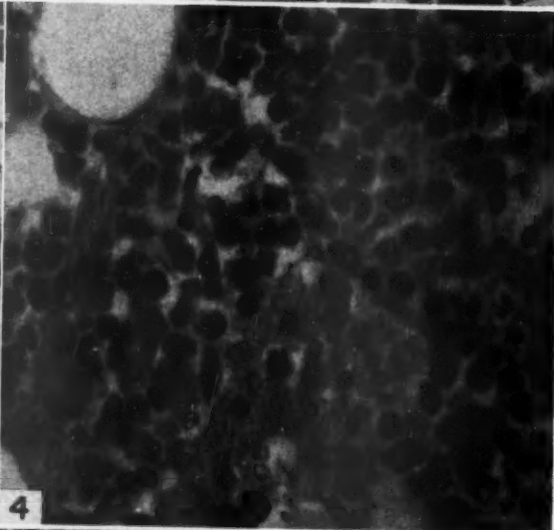
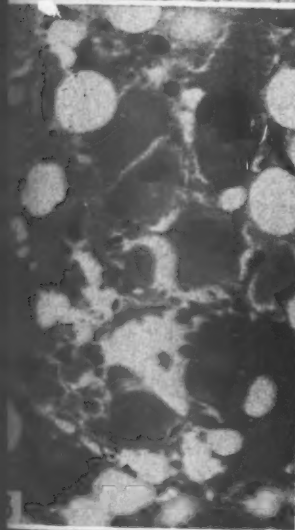
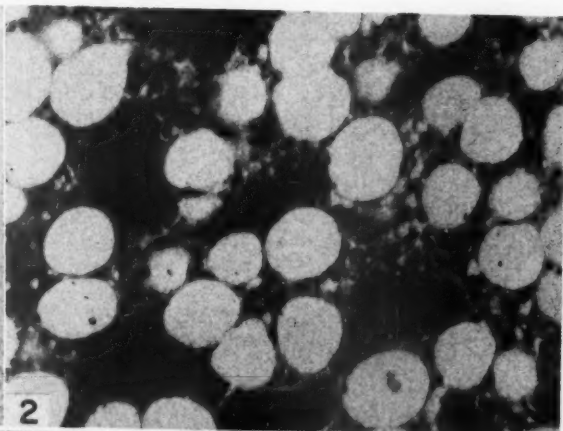
[*Illustrations follow*]

LEGENDS FOR FIGURES

- FIG. 1. Appearance of monkey no. 12, 3 hours before death 9 days after irradiation with 600 r.
- FIG. 2. Bone marrow, 6 days after 800 r. The sinusoids are filled with erythrocytes; no myeloid cells are present, occasional reticulum cells and plasmacytoids are seen. Monkey no. 19. Giemsa's stain. $\times 250$.
- FIG. 3. Bone marrow, 13 days after 600 r., showing an area containing many megakaryocytes; elsewhere the marrow resembled that seen in Figure 2. Monkey no. 34. Giemsa's stain. $\times 250$.
- FIG. 4. Regenerating bone marrow containing immature cells of the erythroid and myeloid series 61 days after exposure to 500 r. Monkey no. 41. Giemsa's stain. $\times 700$.
- FIG. 5. Spleen, 9 days after irradiation with 900 r. A very few lymphocytes remain about a central hyaline mass, marking the remnants of the germinal center of a follicle. The reticulum cells of the pulp are hyperplastic. Monkey no. 2. Hematoxylin and eosin stain. $\times 100$.

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FIG. 6. Section of spleen from an animal that died 12 days after irradiation with 800 r. Lymphocytes have largely disappeared; a ring hemorrhage is seen about the previous site of a germinal center. Monkey no. 64. Hematoxylin and eosin stain. $\times 80$.

FIG. 7. Regenerated follicles in spleen 496 days after irradiation with 600 r. Monkey no. 9. Hematoxylin and eosin stain. $\times 150$.

FIG. 8. Surface of spleen showing follicular hyperplasia 325 days after exposure to 500 r. Monkey no. 42. $\times 5$.

FIG. 9. Erythrophagocytosis by monocyte in lymph node, 12 days after irradiation with 700 r. Monkey no. 32. Hematoxylin and eosin stain. $\times 800$.

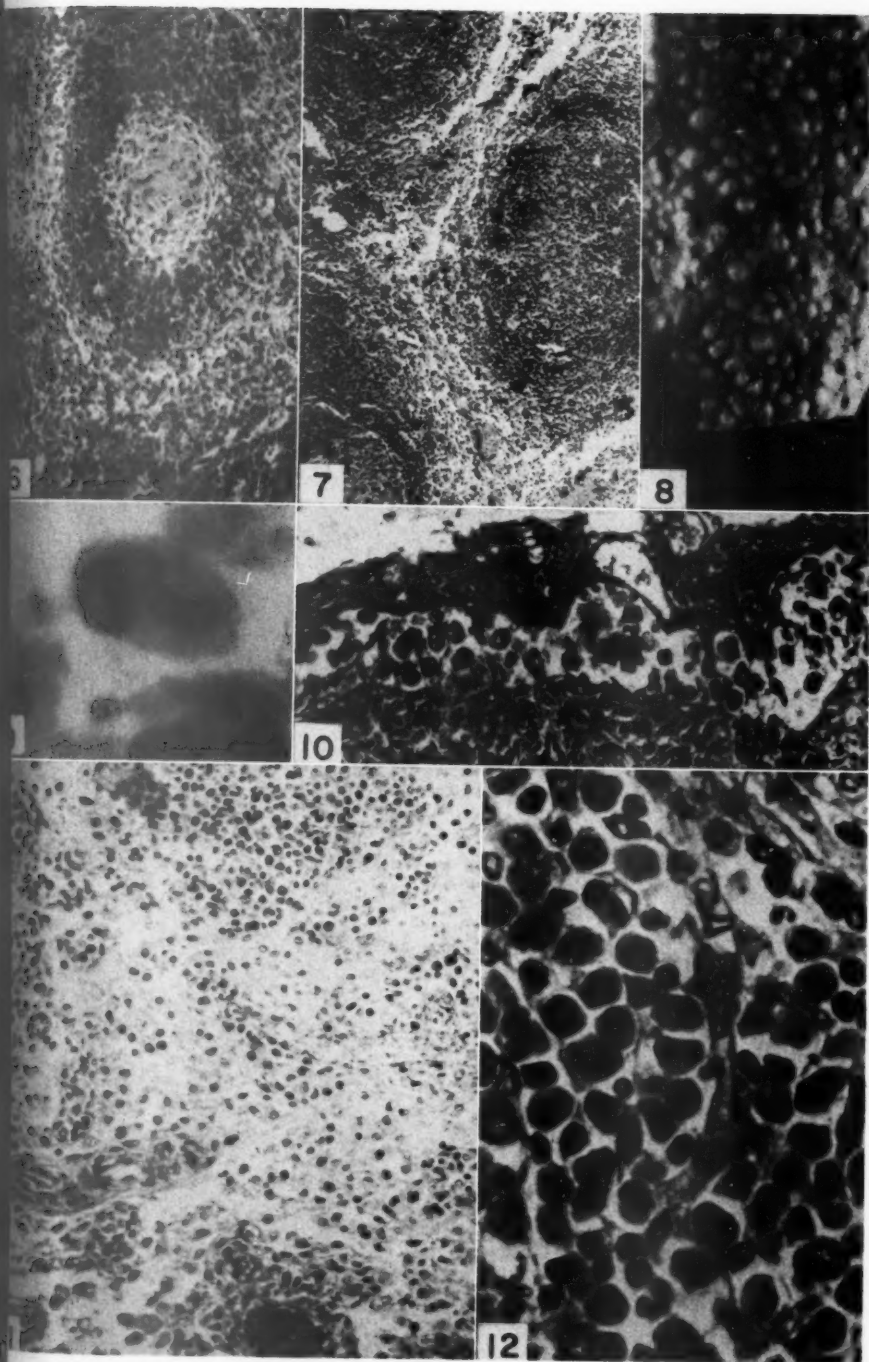
FIG. 10. Large numbers of monocytes in peripheral sinus of a lymph node 8 days after 900 r. Monkey no. 60. Hematoxylin and eosin stain. $\times 200$.

FIG. 11. Loss of lymphocytes and prominence of reticulum cells in lymph node 12 days after 800 r. The small cells that resemble lymphocytes are plasma cells. See Figure 12. Monkey no. 64. Hematoxylin and eosin stain. $\times 100$.

FIG. 12. Plasma cells in lymph node shown in Figure 11. Monkey no. 64. Hematoxylin and eosin stain. $\times 700$.

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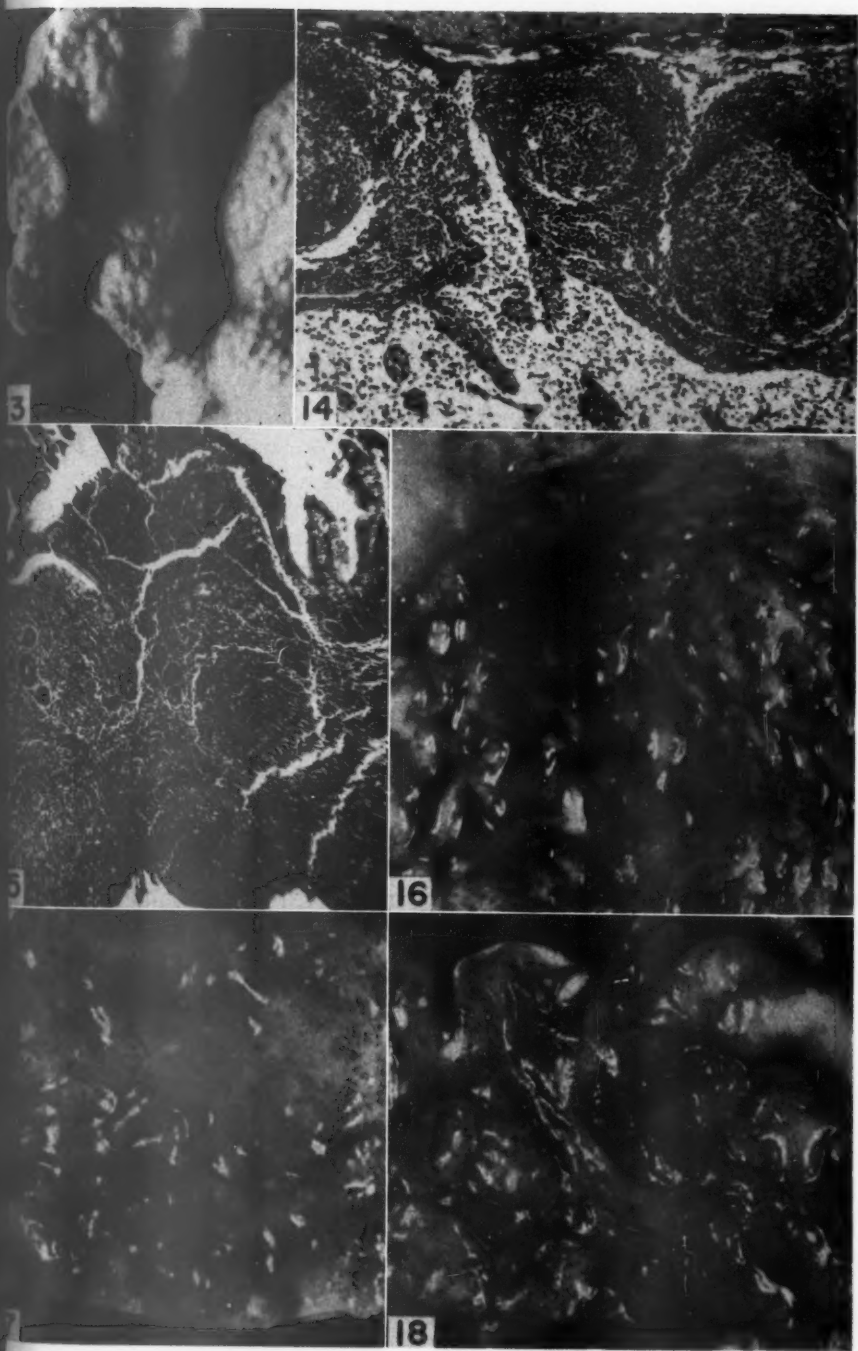
- FIG. 13. Surface of several lymph nodes showing follicular hyperplasia 325 days after exposure to 500 r. Monkey no. 42. $\times 5$.
- FIG. 14. Restitution of follicles in a lymph node 496 days after irradiation with 600 r. Monkey no. 9. Hematoxylin and eosin stain. $\times 150$.
- FIG. 15. Hyperplasia of follicles in a Peyer's patch in the ileum 324 days after 400 r. Monkey no. 46. Hematoxylin and eosin stain. $\times 100$.
- FIG. 16. Petechiae and ecchymoses in gastric mucosa 17 days following irradiation with 600 r. Monkey no. 17. $\times 4$.
- FIG. 17. Petechiae in duodenal mucosa 10 days after 800 r. Monkey no. 5. $\times 3$.
- FIG. 18. Mucosa of cecum bearing many petechiae. On the surface of one of the rugae near the center of the photograph are five small ulcers arranged in linear fashion. Monkey no. 5. $\times 3$.

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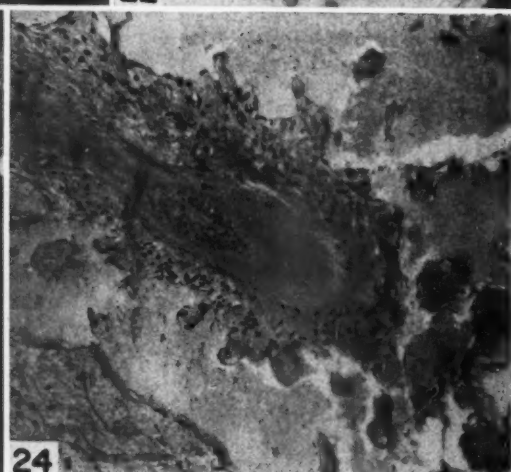
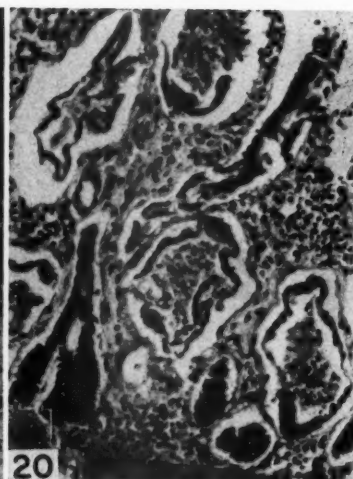
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- FIG. 19. Superficial necrosis of colonic mucosa showing preservation of the deepest portion of many of the glands. Fibrin thrombi in the small vessels beneath the muscularis mucosae. Animal died 9 days after receiving 600 r. irradiation. Monkey no. 12. Hematoxylin and eosin stain. $\times 100$.
- FIG. 20. Obstruction and dilatation of mucosal glands in the colon 183 days following exposure to 600 r. Monkey no. 36. Hematoxylin and eosin stain. $\times 165$.
- FIG. 21. Facial edema due to extensive necrotizing gingivitis 17 days after 600 r. Monkey no. 38.
- FIG. 22. Dissection of animal seen in Figure 21, showing necrotizing gingivitis and noma-like moist gangrene of cheek. Monkey no. 38.
- FIG. 23. Dry gangrene of face following bilateral thrombosis of external maxillary arteries due to necrotizing gingivitis 33 days after 400 r. Monkey no. 40.
- FIG. 24. Section of hair shaft and neighboring tissues from lip of animal shown in Figures 21 and 22, showing the extreme interstitial edema and distention of lymphatics 17 days after exposure to 600 r. Monkey no. 38. Hematoxylin and eosin stain. $\times 100$.

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- FIG. 25. Focal necrosis and ulceration along lateral margin of tongue 22 days following irradiation with 500 r. Monkey no. 101. $\times 4$.
- FIG. 26. Inguinal skin bearing many petechiae 10 days after 800 r. Monkey no. 5. $\times 2$.
- FIG. 27. Epilation of trunk, flanks, upper arms, and scalp 15 days after irradiation with 600 r. Monkey no. 17.
- FIG. 28. Intercellular edema of epidermis and corium 13 days after 600 r. Basal epithelial cells show extensive vacuolization of cytoplasm; nuclei exhibit chromatin clumping. Monkey no. 3. Hematoxylin and eosin stain. $\times 650$.
- FIG. 29. Distortion of hair follicle with swelling and degeneration of sheath cells 17 days after 600 r. Monkey no. 17. Hematoxylin and eosin stain. $\times 650$.
- FIG. 30. Focal edema and hemorrhage in lung 17 days after 600 r. Monkey no. 17. Hematoxylin and eosin stain. $\times 100$.

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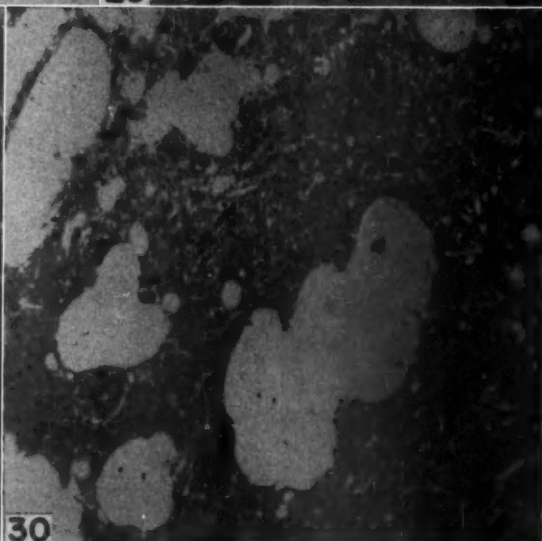
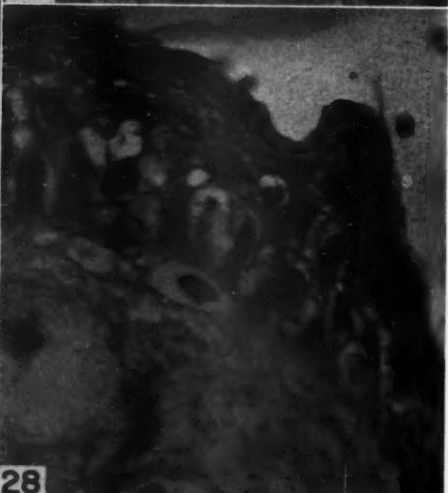
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- FIG. 31. Necrosis of bronchial wall, accumulation of cellular debris; neutrophils are absent. Animal received 500 r. 13 days before death. Monkey no. 13. Hematoxylin and eosin stain. $\times 100$.
- FIG. 32. Seminiferous tubules 10 days after 900 r. Spermatogonia have disappeared leaving only undifferentiated cells. The minute size of the lumen in the tubules is due to immaturity of the animal. A small clump of interstitial cells shows little change. Monkey no. 63. Hematoxylin and eosin stain. $\times 350$.
- FIG. 33. Ova in cortex of ovary showing granularity and shrinking of cytoplasm. Most of the nuclei have disappeared from the ova; the surrounding follicular cells appear uninjured 9 days after 900 r. Monkey no. 61. Hematoxylin and eosin stain. $\times 350$.
- FIG. 34. Multiple ova within single follicles, or blastomeres resulting from parthenogenic cleavage of ova, 16 days after irradiation with 500 r. Monkey no. 119. Hematoxylin and eosin stain. $\times 150$.
- FIG. 35. Epicardial hemorrhages 17 days after exposure to 600 r. Monkey no. 17. $\times 3$.
- FIG. 36. Atrophy of all layers of the adrenal cortex, most pronounced in the zona glomerulosa 396 days after irradiation with 500 r. There is an absence of lipid droplets and loss of trabecular pattern in the fascicular layer. Grossly the adrenal gland was less than half normal size. Monkey no. 44. Hematoxylin and eosin stain. $\times 90$.

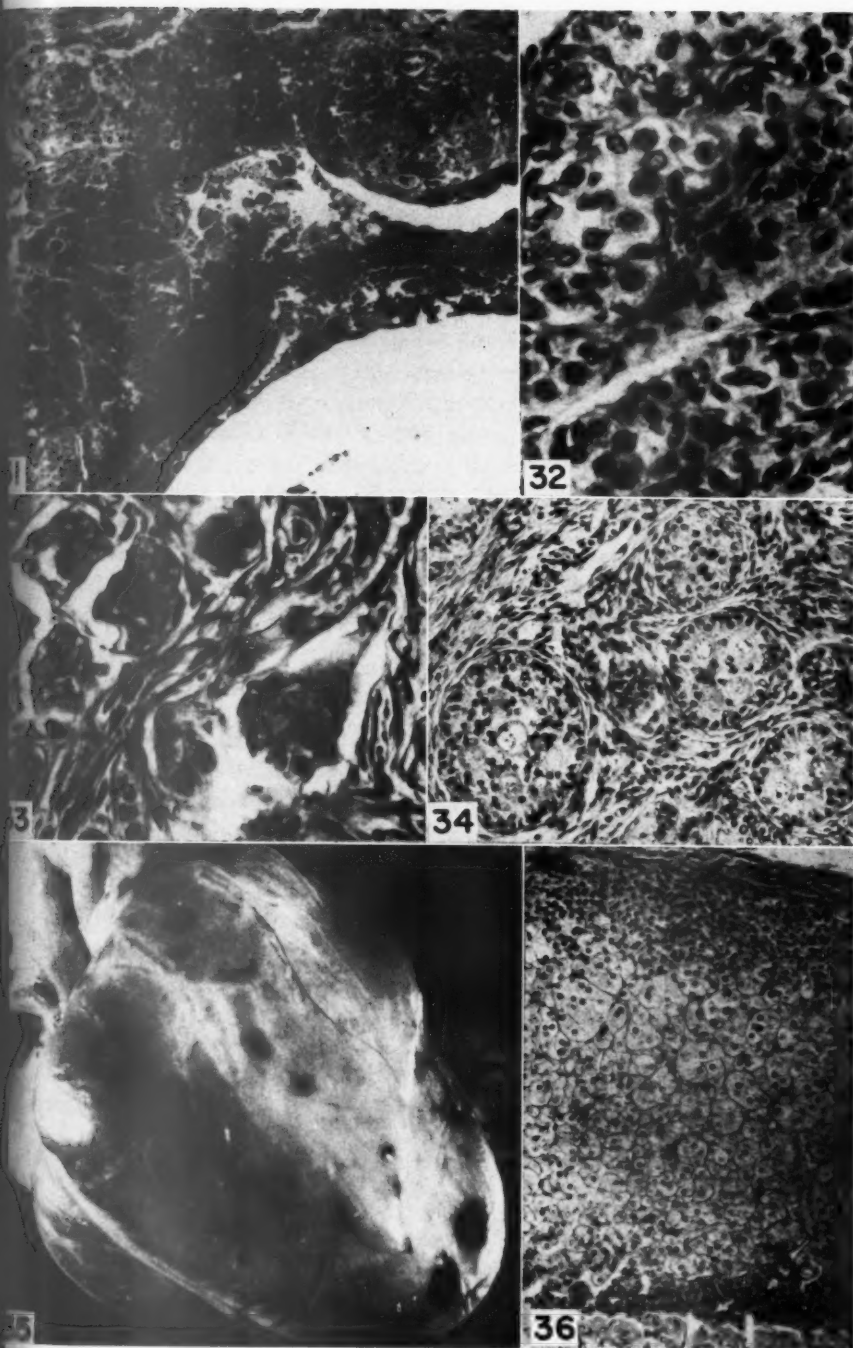
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THE CULTIVATION OF EQUINE ABORTION VIRUS IN CAT TISSUE IN VITRO*

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The cultivation of equine abortion virus in fetal horse lung and spleen *in vitro* has been reported by Randall, Ryden, Doll, and Schell.¹ The work of Dimock *et al.*, who described and characterized the disease, is noted in that publication. Anderson and Goodpasture² presented histologic evidence of infection in Syrian hamsters through three serial passages. After repeated effort over a 5-year period, Doll^{3,4} has recently confirmed and extended this work, reporting the adaptation of a number of isolates of abortion virus to hamsters of different ages. In a recent study,⁵ in which the suspended cell method was utilized, tissues from pig, cow, sheep, mouse, cat, hamster, and guinea-pig embryos and day-old dog, hamster, rat, and rabbit were inoculated with equine abortion virus. In primary passage, intranuclear inclusions were observed in tissues of embryonic cat and hamster, and day-old hamster, dog, and rabbit. On serial passage this virus could be propagated only in cat and embryonic hamster tissue. In this communication the completed studies with cat tissues are reported.

MATERIAL AND METHODS

Tissues. Domestic cats of varying stages of gestation were anesthetized with ether and the uterus and contents removed aseptically. Early in the study, tissues of day-old cats were utilized and three passages were made in their tissues, but because of possible contamination with feline panleukopenia virus, these efforts were abandoned; otherwise their tissues were as satisfactory as embryonic tissues in all respects. Because of previous success in cultivating the virus in lung and spleen of the fetal horse, these organs were removed aseptically from the cat embryo, minced into fragments 2 to 3 mm. in width, and rinsed until clear with Earle's balanced salt solution.

Tissue Culture Materials. The nutrient fluid employed consisted of 75 per cent human ascitic fluid and 25 per cent Earle's solution. Sterility of all materials was controlled by the usual bacteriologic techniques.

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Virus. The infected tissue was obtained from a typical field case of virus abortion. Infected lung, rich in inclusion bodies, was frozen and ground, diluted 1:2 with physiologic saline solution, and centrifuged at 13,500 r.p.m. for 30 minutes at 4° C. The resulting clear, bacteriologically sterile supernatant was stored in ampules at -30° C., and is referred to as standard stored virus.

Techniques of Cell Maintenance. The tissues of choice, spleen and lung, were distributed in flasks. For each type of tissue and for each serial passage, nine flasks were arranged, three in each series serving as controls. All flasks were incubated for 3 to 4 days at 37° C. Twenty to thirty pieces of tissue were transferred by a curved pipette to a rubber-stoppered 50 ml. Erlenmeyer vessel containing 3 ml. of nutrient fluid, pH 7.6 to 7.8. In addition to the routine procedures, a large number of flasks containing 75 mg. of lung were prepared for the 14th and 15th passage. Initially, the inoculum consisted of 0.1 ml. of a 50 per cent suspension of standard virus. Subsequent sets of flasks were inoculated with passage virus which was prepared by pooling the cultured tissue and freezing and grinding to a smooth paste. The resulting material was mixed with like passage nutrient fluid, adjusting the amount so that each serial passage was diluted by a factor of 1:33. Between passages the material was stored in ampules at -30° C. Representative pieces of tissue from both test and control flasks from each passage were fixed in Zenker-acetic acid and stained with hematoxylin and eosin.

Experimental Animals. In order to determine the infectivity of the 15th cat passage virus, mares of 283 and 304 days' gestation, designated mare I and II respectively, were inoculated intravenously with 10 ml. of tissue culture material containing approximately 187 mg. of fetal cat lung. In addition, inoculations were made directly into the equine fetus by the method described by Doll.⁶ Each fetus received 20 ml. of inoculum, representing 374 mg. of lung. Serum was collected from each mare before inoculation and subsequently at intervals of 1 and 2 weeks. All samples were stored in the deep freeze at -18° C. until ready for use.

Complement Fixation Methods. The original complement fixation method for equine virus abortion, employing the 50 per cent hemolysis end-point technique, was described by Randall, McVickar, and Doll.⁷ The procedure requires relatively large amounts of reagents. In the present communication the more conventional method was utilized because of the smaller amounts of test material required. This modification has been documented in detail⁸ and the methods outlined have been rigidly adhered to in this report.

EXPERIMENTAL RESULTS

Propagation of Virus in Serial Passage. The various flask preparations maintained for 3 to 4 days showed a change in pH from 7.6 to 7.0 on the average. No appreciable difference between test and control cultures was noted. Lung and spleen from each serial passage were harvested in 3 to 4 days (Figs. 1 and 2). In the initial experiment eosinophilic intranuclear inclusions were observed in moderate numbers in the lining cells of the bronchi and in cells of the interstitium of the lung. Inclusions were noted in each passage; in some they were exceptionally abundant, in others they were scarce. Inclusions were noted in splenic tissue in each passage in few to moderate numbers. They occurred in reticulo-endothelial cells. Inclusions in both tissues could not be distinguished from those previously described.^{1,2} Necrosis was present in both spleen and lung in some sections, but seemed unrelated to the presence of infection and was noticed in the controls as often as in the test preparations. The original virus had been diluted 1:10²⁴ as a result of serial transfer through the 16th passage.

Infectivity of Tissue Culture Virus. Following inoculation of the mares by intravenous and *in utero* routes with 15th passage material, each mare aborted on the 5th day. The abortions were typical of those resulting from infections with equine abortion virus, neither mare showing symptoms of impending abortion or illness. Fetal tissues were fixed in Zenker-acetic acid and stained with hematoxylin and eosin.

The gross and microscopic lesions, some of which are shown (Figs. 3 and 4), of the equine fetuses receiving the tissue culture inoculum were identical with those occurring in other fetuses (horse) inoculated with abortion virus obtained from typically infected equine fetuses as previously described by Doll.⁶ Similar lesions occur in equine fetuses born by abortion as a result of either intravenous inoculation or natural infection. The immune status of the mares apparently provided no deterrent to infection of the fetus by direct inoculation because no passive immunity is obtained by the equine fetus *in utero*.^{6,9}

Other experiments were designed to determine the infectivity for horse tissue of the virus propagated in tissue cultures. Fetal horse lung and spleen maintained by the same flask method as the cat tissue were inoculated with the 2nd, 3rd, 13th, and 14th cat passage material, incubated for 3 to 4 days, and stained by hematoxylin and eosin. Numerous intranuclear inclusions were seen in the lining cells of the bronchi, in the interstitial cells of the lung, and in reticulo-endothelial cells of the spleen in every case. These same effects were observed in

cultures of cat tissue when the infected horse tissue was used as inoculum.

Antibody Response to Inoculation of Tissue Culture Virus. Both mares had been given intravenous inoculations of active abortion virus from equine fetuses 11 to 12 months prior to inoculation with tissue culture virus. Each should have been immune to abortion by intravenous inoculation.⁴ Following inoculation with cat tissue culture virus, complement fixation tests on sera from the mares showed an increase of antibody in the case of mare I from a pre-inoculation titer of 1:16 to 1:64 2 weeks after inoculation. Mare II had a pre-inoculation titer of 1:32 and no increase in antibody response could be demonstrated after 2 weeks.

Complement Fixing Activity of Tissue Culture Virus. The cat tissue maintained *in vitro* proved highly anti-complementary. Heating, centrifugation, and dilution failed to remove this anti-complementary factor. Due to the anti-complementary nature of the antigen, no conclusions could be drawn from the results.

DISCUSSION

It has been assumed that the virus of equine abortion is restricted in its host range. It is clear, however, that in addition to the horse the virus can be propagated in the Syrian hamster and, according to the present communication, in cat tissue maintained *in vitro*. It is odd that the virus in the 15th passage failed to infect suckling kittens¹⁰ by intraperitoneal, intravenous, intrathoracic, and subcutaneous routes. Blind serial passage through three litters of day-old kittens failed to show any evidence of infection and horse lung and spleen maintained *in vitro* and inoculated with ground kitten tissue failed to develop inclusions. Blind passage of the standard virus through two litters of suckling kittens was equally unrewarding.

The question arises as to a possible relationship of the virus propagated in cat tissue to feline panleukopenia. Preliminary¹⁰ studies employing cat and fetal horse tissues maintained *in vitro* and inoculated with a known infectious strain of feline panleukopenia virus failed to show inclusions in tissue of either type.

SUMMARY

Cat lung and spleen maintained *in vitro* have been shown to support the propagation of the virus of equine abortion through sixteen serial passages. Characteristic intranuclear inclusions were observed in tissues from each passage. Cat tissue from the 15th passage, representing

a dilution of the original inoculum of $1:10^{23}$, caused mares to abort. The lesions in the fetuses were characteristic of the disease seen in horses.

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[Illustrations follow]

LEGENDS FOR FIGURES

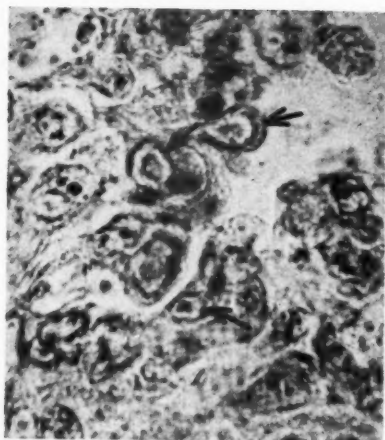
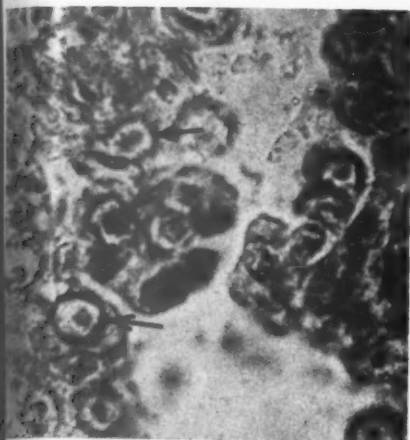
- FIGS. 1 and 2. Inoculated fetal cat lung (16th serial passage) maintained for 4 days by the flask method, fixed in Zenker-acetic acid, and stained with hematoxylin and eosin. Arrows indicate a few of the inclusions (many of which are out of focus), occurring in cells of the parenchyma. $\times 727$.
- FIG. 3. Lung of fetal horse, from abortion following inoculation of the 15th serial passage of equine abortion virus propagated in cat tissue, fixed in Zenker-acetic acid and stained with hematoxylin and eosin. A medium-sized bronchus shows ulceration and plugging of the lumen with debris and mononuclear cells. $\times 150$.
- FIG. 4. Pulmonary tissue from the same source as used for Figure 3. A small projection of hyperplastic bronchial epithelium contains many inclusions which are characteristic. The intensely basophilic chromatin material is margined around the periphery of the nucleus in an irregular fashion. Oil immersion. $\times 727$.

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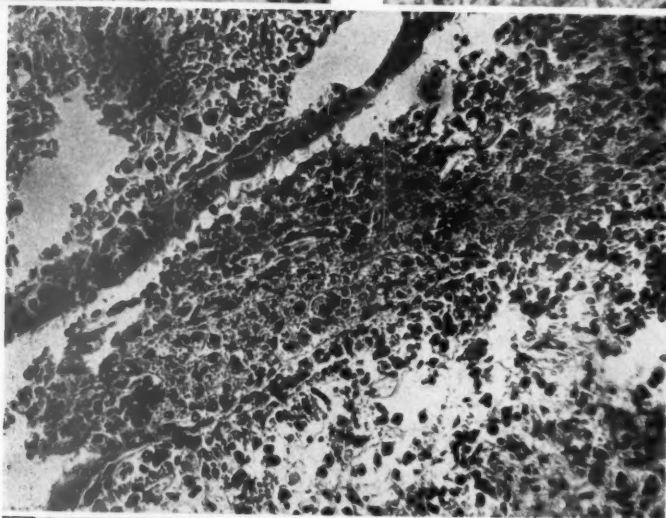
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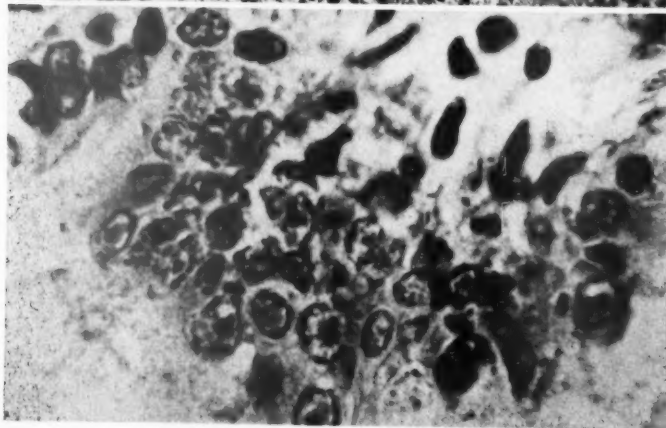
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THE VIRAL RANGE IN VITRO OF A MALIGNANT HUMAN EPITHELIAL CELL (STRAIN HELA, GEY)

I. MULTIPLICATION OF HERPES SIMPLEX, PSEUDORABIES, AND VACCINIA VIRUSES *

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(From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.)

Human epithelial cells derived from an epidermoid carcinoma of the cervix (strain HeLa, Gey) have been successfully used for studies *in vitro* with poliomyelitis virus.^{2,3} These cells were isolated in culture in February, 1951, by Dr. G. O. Gey and coworkers⁴ at Johns Hopkins University. In May, 1952, Dr. Gey kindly supplied our laboratory with two roller tube cultures containing explants of strain HeLa cells. Subsequently, procedures were developed at the University of Minnesota for handling these cells in suspension to permit transfer, enumeration, and the preparation of replicate cultures *en masse*.^{2,3} These cultures found ready and efficient application for the isolation and typing of field strains of poliomyelitis virus.^{2,3} Soon after strain HeLa cells were found to support the propagation of poliomyelitis virus, the possibility was recognized that they also might support the growth of other viruses to result in cellular destruction resembling that from infection by poliomyelitis virus.¹ Thus, to aid differentiation of viruses in cultures of strain HeLa cells, it was important to learn the response of the cells to other viruses.

A series of experimental studies was therefore carried out to learn the "viral range" of strain HeLa cells, *i.e.*, the spectrum of viruses capable of infecting these cells *in vitro*. This first paper records the findings that resulted from a study of the viruses of herpes simplex, pseudorabies, and vaccinia. These three viruses are known for their epitheliotrophic properties and for their ability to produce readily recognizable inclusion bodies.

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

Presented in part as a preliminary report¹ before the Society of American Bacteriologists, San Francisco, August, 1953.

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The second paper in this series: *II. Studies with Encephalitis Viruses of the Eastern, Western, West Nile, St. Louis, and Japanese B Types*, follows in this issue. The third article: *III. Studies with Pseudolymphocytic Choriomeningitis Virus*, will appear in the January-February issue, and will be accompanied by a general discussion of the material presented in all three papers.—Editor.

† John and Mary R. Markle Scholar in Medical Science.

MATERIALS AND METHODS

Viruses. The strains of viruses employed for these studies have been described.^{5,6}

The herpes simplex virus⁵ for experiment 2 was contained in supernatant fluid harvested from the 18th passage in strain L fibroblasts; the intracerebral infectivity titer for mice was $10^{-4.7}$ per 0.03 to 0.05 ml. The herpes virus for experiment 3, in a 5 per cent suspension of infected mouse brain, was made up in 5 per cent dextrose in glass distilled water; its intracerebral infectivity titer for mice was $10^{-5.9}$ per 0.03 to 0.05 ml.

Pseudorabies virus (Aujeszky strain)⁶ was used as the supernatant fluid from a 10 per cent mouse brain suspension; the LD₅₀ was $10^{-4.8}$ for mice intracerebrally, 0.03 to 0.05 ml.

The vaccinia virus⁶ in 5 per cent suspension of infected chorioallantoic membrane had an infectivity titer for rabbits of $10^{-8.3}$ per 0.2 ml. intradermally.

The viruses were stored at -70°C . with solid carbon dioxide.

Assays for Viruses. The methods for quantitative infectivity measurements of herpes simplex and pseudorabies viruses in mice and of vaccinia virus in rabbits have been described.^{5,6} Titrations were performed in cultures of strain HeLa cells from 5 to 14 days old. Just before inoculation of virus, the cells in each cultural tube were washed to remove residual virus inhibitor or antibody. All liquid was removed from each tube, care being exercised not to disturb the cells on the glass. Maintenance solution (MS), or balanced salt solution, Hanks's (H), 0.5 ml., was placed in each tube, and the tubes individually tilted to allow the solution to flow over the cells and glass wall. The MS or H was removed and the washing repeated. After removal of the second wash fluid, 0.4 ml. aliquots of serial tenfold dilutions of viral material prepared in a mixture of chicken serum (CHS), 10 per cent, and MS, 90 per cent (CHS-10, MS-90) containing 50 units per ml. of penicillin and 50 μg . per ml. of streptomycin, were transferred to duplicate cultures. The washing procedure was modified as specified for certain experiments by the use of 1.0 ml. aliquots of MS or H and by the performance of three washings.

Strain of Cells. Malignant human epithelial cells, strain HeLa, were employed. This cell strain, originally supplied by Dr. G. O. Gey, has been described.¹⁻⁴

Methods for Cellular Cultivation. The procedures for cultivation of strain HeLa cells have been published.²⁻⁴ For these studies, cells for stock cultures were grown on the dependent walls of square 200 ml. screw cap bottles placed in a horizontal position with a nutritive mixture of adult human serum (HAS), 40 per cent, and balanced salt solution, Hanks's (H), 60 per cent, that contained 50 units per ml. of penicillin and 50 μg . per ml. of streptomycin. Chicken embryonic extract (EE), 2 per cent, was included in the mixture whenever it was desired to increase the rate of cellular multiplication. The cultures were incubated at 36°C . until cells covered most of the glass surface; frequently thereafter the cells were kept at 30°C . To prepare cultures in test tubes for viral studies, aliquots of cellular suspension, prepared from stock cultures, were transferred to tubes with sterile equipment, as follows: (a) removal of the liquid from a bottle; (b) addition of 8 ml. of trypsin,* 0.5 per cent in MS-100, at pH 7.4 to 7.6; (c) incubation of the cells in the trypsin solution at 36°C . for 60 to 90 minutes; (d) dispersion of cells in the suspension by forceful filling and emptying of a 5 or 10 ml. serologic pipette; (e) transfer of the suspension to a round bottom test tube for centrifugation at 1000 r.p.m. for 5 to 10 minutes; (f) removal of the supernatant trypsin solution; (g) resuspension of the cells in HAS-40 to 60, EE-2, H-58 to 38; (h) enumeration of the cells as described^{2,3}; (i) further dilution of the suspension with medium to obtain the desired concentration of cells; and, finally, (j) transfer of aliquots of suspension to

* Difco Laboratories, Detroit, Michigan, trypsin 1:250.

16 by 125 mm. screw cap tubes by employment of a modified Cornwall pipetting apparatus.³ The quantities of cells and medium were set at 30,000 cells per 0.5 ml. of HAS-60 or 50, EE-2, H-38 or 48 per tube, to make cultures available for virus work in from 5 to 10 days after preparation, without further addition of medium. Incubation of tube cultures was carried out in a stationary position at 36° C. for from 4 to 7 days; thereafter, usually at 30° C. Each tube was slanted to permit cells to settle on the dependent glass surface of the lower one third to one half of the tube. For photography, Porter flasks were inoculated with 60,000 cells, or more, per 1.0 ml. of medium to provide cells on a flat glass surface.

Methods for Viral Cultivation. Virus inhibitors were removed from the cultures by washing as described under "Assays for Viruses." When the interval for virus passage at 36° C. was from 5 to 7 days, the quantity of CHS-10, MS-90 required was 0.5 ml.; for from 7 to 10 days, 1.0 ml.; and for more than 10 days, the old medium was replaced by fresh CHS-10, MS-90 to prevent degenerative changes from malnutrition. A stock of "washed" cultures in CHS-10, MS-90 was kept at 30° C. for use within a period of several days after washing. The inoculum of virus was 0.05 ml. for each tube containing 0.5 ml. of medium, and 0.1 ml. for each tube with 1.0 ml. The CHS-10, MS-90 contained 50 units per ml. of penicillin and 50 µg. per ml. of streptomycin.

Photographic Methods. Unstained cells in Porter flasks were photographed, after removal of the liquid medium; on commercial orthochromatic film, 3¼ by 4¼ inches, by the employment of an American Optical Co. lamp, no. 735, a 16 mm. apochromatic objective, and a Micam photomicrograph camera (C. Leitz, Inc.).

Cells stained with hematoxylin and phloxine after growth on coverglasses, 11 by 22 mm., in Porter flasks also were photographed.

EXPERIMENTAL RESULTS

The experiments with each virus were planned to learn (a) whether destruction of strain HeLa cells occurred, and (b) whether virus multiplied.

Herpes Simplex Virus

Cytologic Effects of Herpes Simplex Virus. The destructive effects of herpes simplex virus for strain HeLa cells became apparent within 2 days after the inoculation of virus. For photographic purposes a passage of virus was performed in Porter flask cultures (experiment 1).

Experiment 1. The cells and glass surfaces of four Porter flasks were washed twice with MS-100, 1.0 ml., herpes simplex virus, 0.1 ml., from the 16th passage of experiment 2 and the medium, CHS-10, MS-90, 1.0 ml. were added to each of two flasks; the two control cultures received only medium. The fluid was removed from each flask before the cells were photographed; it was replaced after photography when further incubation was desired. Photographs of cells from this experiment are shown in Figures 1 to 4.

The destructive effects of herpes simplex virus for strain HeLa cells are presented in Figures 1 to 4. The cells in the control, non-infected cultures (Fig. 1) were polygonal. Occasional round forms resulted from mitosis, crowding of cells, and the slight degree of spontaneous degeneration that occurs in all cellular cultures. The nuclei and nucleoli were seen distinctly. In contrast to these normal cells, cells 1 day after

infection with herpes simplex virus had undergone changes (Fig. 2). These cells were now round and gathered in small clumps; distinct nucleoli were less evident. Two days after viral inoculation, most of the cells were round and reduced in size; large clumps of cells were present; few nucleoli could be seen. Many nuclei were sharply outlined by peripheral chromatin material and dead cells were present (Fig. 3).

TABLE I
Propagation in Vitro of Herpes Simplex Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture	Cumulative log of dilution of original viral inoculum	Results as indicated by		
			Cytopathogenic effect	Infectivity log of cultural fluid for	
				Mice*	HeLa cells†
Inoculum				4.7	
1	3	1.0	2/2‡		
7	19	7.0	2/2		3.0
8	21	8.0	2/2	4.8	2.0-3.0
10§	26	10.0	2/2		
15	44	15.0	2/2	4.6	
16	46	16.0	2/2	5.4	4.0-5.0

* The results of mouse titrations in all tables are expressed as the negative log of the LD₅₀/0.05 ml. of diluted cultural liquid.

† The results of strain HeLa titrations are given as the negative log of the dilution of cultural liquid which, per 0.4 ml., produced a specific viral cytopathogenic effect in a tube culture of cells after 4 to 6 days of incubation at 36° C.

‡ For each of the tables, the numerator signifies the number of cultures that showed a viral cytopathogenic effect. The denominator indicates the number of cultures inoculated with virus.

§ The identity of the virus was established by neutralization of its infectivity for mice with specific antibodies.

The appearance of normal cells in control cultures (Fig. 4) was in sharp contrast to that of cells damaged by virus (Fig. 3).

Distinct foci of degeneration were occasionally seen in cultures of strain HeLa within 1 to 3 days after inoculation of small quantities of virus (Fig. 5). These areas were considered to be analogous to plaques from bacteriophagy. However, since the spread of virus in these cultures was not delimited by a solid material such as agar, the destructive process eventually extended beyond the focal areas to involve the entire cellular population.

Multiplication of Herpes Simplex Virus. Two experiments were done to learn whether herpes simplex virus from different source materials multiplies in cultures of strain HeLa cells. The first experiment employed tissue culture virus.

Experiment 2. Herpes simplex virus, 0.05 ml., from a culture of strain L cells was inoculated into each of two tube cultures of strain HeLa cells made ready for viral cultivation by washing and the addition of CHS-10, MS-90, 0.5 ml. When degeneration of cells had occurred, the supernatant liquids from the two tubes were pooled, and aliquots, 0.05 ml., were transferred to each of two uninfected cultures to effect serial passage of virus. The data from experiment 2 are given in Table I.

The results of experiment 2 (Table I) show that herpes simplex virus multiplied rapidly in cultures of strain HeLa cells. Virus, infectious for mice and destructive for strain HeLa cells, was present after 16 serial virus passages, over a period of 46 days. These passages resulted in a dilution of original virus of 10^{16} . The infectivity for mice of virus from the 10th passage was neutralized by specific antibodies. It may be noted that the titration values for virus were lower for strain HeLa cultures than for mice. These differences in titration end points may reflect an incomplete removal of serum viral inhibitors from the HeLa cultures even though calculation revealed that the human serum in each tube had been diluted, by washing, for the 8th passage titration approximately 1:1200, and for the 16th passage measurement, approximately 1:96,000.

Another experiment was effected by using virus from an animal source in an attempt to confirm the results of experiment 2.

Experiment 3. The procedure employed for experiment 3 was identical with that for experiment 2 except that herpes simplex virus from infected mouse brains was used to start the serial passages. The results of experiment 3 are shown in Table II.

The data presented in Table II show that herpes simplex virus from

TABLE II
Propagation in Vitro of Herpes Simplex Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture	Cumulative log of dilution of original viral inoculum	Results as indicated by		
			Cytopathogenic effect	Infectivity log of cultural fluid for	
				Mice	HeLa cells*
Inoculum				5.9	
1	3	1.0	2/2		
8†	23	8.0	2/2		3.0-4.0
9	26	9.0	2/2		3.0
15	43	15.0	2/2		

* The results of strain HeLa titrations are given as the negative log of the dilution of cultural liquid, which, per 0.4 ml., produced a specific viral cytopathogenic effect in a tube culture of cells after 6 days of incubation at 36° C.

† The identity of the virus was established by the neutralization of its infectivity for mice with specific antibodies.

infected mouse brains propagates readily in cultures of strain HeLa. Fifteen serial passages were carried out over a 43-day period resulting in a dilution of the original viral inoculum (10^{15}) that far exceeded the LD_{50} ($10^{-5.9}$) of the source mouse brain suspension. Quantitation of virus at the eighth and ninth passages revealed titers of $10^{-3.0}$, or more.

To learn whether intranuclear inclusions were present in strain HeLa cells infected with herpes simplex virus, cells were stained (experiment 4).

Experiment 4. The cells and glass surfaces of six Porter flasks with cellular growth on coverglasses, 11 by 22 mm., were washed twice with MS-100, 0.5 ml. The mixture, CHS-10, MS-90, 0.75 ml., was added to each flask. Herpes simplex virus, 0.1 ml., from cultures of strain L fibroblasts was inoculated into four flasks; two flasks served as virus-free controls. After 2, 3, and 5 days of incubation at 36° C., cells were stained with hematoxylin and phloxine. Photographs of stained cells are presented in Figures 6 and 7.

Acidophilic, intranuclear inclusion bodies of type A were found in many cells from strain HeLa cultures which had been inoculated with herpes simplex virus (Figs. 6 and 7). Large inclusions were seen often in multinucleated cells (Fig. 6).

Pseudorabies Virus

Two passage series, series A and B, were carried out with strain HeLa cells and pseudorabies virus. The descriptive material is limited for the most part to series A, experiments 5 to 7.

Cytologic Effects of Pseudorabies Virus. Destruction of strain HeLa cells was seen during the first passage of pseudorabies virus. To permit recording of these cytologic changes photographically, experiment 5 was performed in Porter flasks.

Experiment 5. The procedure for this experiment was identical with that used for experiment 1. Pseudorabies virus, 0.1 ml., from the 13th passage of virus in experiment 6, was employed. The findings are recorded in Figures 8 to 12.

The destructive effects of pseudorabies virus for strain HeLa cells are shown in Figures 8, 10, and 12. The first cytologic changes in experiment 5 were observed on the third day after viral inoculation when focal degeneration of cells became evident (Fig. 8). Indeed, localized areas of cellular destruction resulted in all experiments from infection by pseudorabies virus, but such areas were not observed in the uninfected control cultures (Fig. 9). Frequently these focal areas could be clearly seen macroscopically (Fig. 12).

The common occurrence of foci of degeneration in cultures of pseudorabies virus is thought to be related to the slow rate at which the destructive process progressed. Each focal area enlarged slowly over a period of several days. With time, however, new foci developed and coalesced to result in nearly total cellular destruction (Fig. 10). Cells in control cultures retained a normal appearance (Fig. 11). These observations were interpreted as evidence of a comparative resistance of strain HeLa cells to pseudorabies virus since infection of cells occurred most frequently where large quantities of virus existed, *i.e.*, near areas of cellular degeneration.

Multiplication of Pseudorabies Virus. An attempt was made by serial passage of virus to learn whether pseudorabies virus propagates in strain HeLa cells (experiment 6).

Experiment 6. The procedure employed for experiment 6 with pseudorabies virus was similar to that used for experiment 2 with herpes simplex virus. The data from experiment 6 are presented in Table III.

It is apparent from the results of experiment 6 (Table III) that strain HeLa cells supported the growth of pseudorabies virus over a 79-day period. Fifteen serial passages of virus resulted in a dilution

TABLE III
Propagation in Vitro of Pseudorabies Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture	Cumulative log of dilution of original viral inoculum	Results as indicated by		
			Cytopathogenic effect	Infectivity log of cultural fluid for	
				Mice	HeLa cells*
Inoculum					
1	5	1.0	2/2	3.8	
5	37	5.0	2/2		2.0-3.0
10	59	10.0	2/2		2.0-3.0
13	72	13.0	2/2	4.7	3.0-4.0
15	79	15.0	2/2		

* The results of strain HeLa titrations are given as the negative log of the dilution of cultural liquid, which, per 0.4 ml., produced a specific viral cytopathogenic effect in a tube culture after 7 days of incubation at 36° C.

of the original viral inoculum of 10^{15} times. It is of interest that comparative titrations in mice and in strain HeLa cells of virus from the 13th passage in strain HeLa yielded a higher value for the mouse titration.

Evidence of adaptation of pseudorabies virus to growth in these human cells was noted. During passages 2 to 5, the time required for destruction of cells was from 7 to 9 days, whereas from the 6th to 15th passages, this time shortened to from 2 to 6 days.

To permit the staining of cells infected with pseudorabies virus, experiment 7 was carried out.

Experiment 7. Porter flask cultures with cells on coverglasses were used as in experiment 4. Pseudorabies virus, 0.1 ml., as a 10 per cent suspension of infected mouse brain, was placed in each of four flasks. Cells were stained on days 3, 5, and 6 after inoculation of virus. A photograph of stained cells is presented in Figure 13.

Intranuclear, acidophilic, type A inclusion bodies of pseudorabies virus were found in cells at the edge of foci of cellular degeneration (Fig. 13). The frequency of occurrence of pseudorabies inclusions in strain HeLa cells was considerably less than that from infection by herpes simplex virus. This observation again may reflect the relatively greater resistance of strain HeLa cells to infection by pseudorabies virus than by herpes simplex virus.

The experimental findings for pseudorabies virus in HeLa cellular cultures were confirmed fully by results from a second parallel passage series, series B. This series was initiated with an inoculum of 0.1 ml. of supernatant fluid from passage 6 of series A; it was maintained thereafter for 17 more transfers which resulted in a minimal dilution factor for the inoculum of 10^{17} . The cytologic changes were as noted for series A.

Vaccinia Virus

Cytologic Effects of Vaccinia Virus. Degenerative changes in strain HeLa cells were seen upon the first passage of vaccinia virus. These changes were recorded photographically in Porter flask cultures (experiment 8).

Experiment 8. The procedure for experiment 1 was followed. Virus from the 14th passage of experiment 9 was employed. The results are shown photographically in Figures 14 to 16.

The destructive effects of vaccinia virus upon strain HeLa cells were seen to occur as early as 24 hours after the inoculation of virus (Fig. 14). Cells were round and clumped. By the second day the changes were more marked (Fig. 15) and by the fourth day, only scattered degenerate cells remained on the glass wall of the flask (Fig. 16). Cells from control cultures without virus are shown in Figures 4 and 11 for comparison with the infected cells seen in Figures 15 and 16.

Multiplication of Vaccinia Virus. An experiment was performed to

learn whether vaccinia virus would propagate when passed serially in cultures of strain HeLa.

Experiment 9. The procedure employed for experiment 9 was similar to that for experiment 2. Vaccinia virus, 0.05 ml., from infected chorioallantoic membranes was inoculated into each first passage culture. The data from this experiment are shown in Table IV.

It is apparent from the results of experiment 9 (Table IV) that vaccinia virus multiplied in cultures of strain HeLa cells. The cytopathogenic effect of this virus persisted through fifteen passages of virus (10^{15} dilution of original virus) over a 39-day period. Titration values in rabbits and in strain HeLa cultures for virus from selected passages also provided evidence that virus multiplied.

TABLE IV
Propagation in Vitro of Vaccinia Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture	Cumulative log of dilution of original viral inoculum	Results as indicated by		
			Cytopathogenic effect	Infectivity log of cultural fluid for	
				Rabbits*	HeLa cells†
Inoculum				7.0	
1	1	1.0	2/2		
4	10	4.0	2/2	5.0	3.0-4.0
6	15	6.0	2/2		4.0-5.0
8	20	8.0	2/2	4.6	
11	28	11.0	2/2	5.3	5.0
15	39	15.0	2/2		

* The results of titrations intradermally in rabbits are expressed as the negative log of the MID₅₀/0.2 ml. of diluted cultural liquid.

† The results of strain HeLa titrations are given as the negative log of the dilution of cultural liquid which, per 0.4 ml., produces a specific viral cytopathogenic effect in tube cultures after 5 to 6 days of incubation at 36° C.

To learn whether cytoplasmic inclusion bodies occurred in cells from strain HeLa infected with vaccinia virus, cells were grown on cover-glasses in Porter flasks and stained.

Experiment 10. Five protocols, as described for experiment 4 with herpes virus, were completed. The vaccinia virus for protocols A and B was derived from infected chorioallantoic membranes, and for protocols C, D, and E, from passage 13 of experiment 9. The procedure for protocol D was modified by incubation at 30° C. and that for protocol E by the use of MS-100 instead of CHS-10, MS-90. Photographs of cells from these cultures are presented in Figures 17 and 18.

The attempts in protocols A and B of experiment 10 to produce inclusions in strain HeLa cells at 36° C. failed even though marked

cellular destruction occurred; a similar attempt in protocol C resulted in only a few cells containing Guarnieri bodies. Therefore, in an effort to produce a more widespread occurrence of inclusions, the physiologic activity of the cells was lowered. The method employed for lowering cellular metabolic activity was either to decrease the temperature of incubation to 30° C. (protocol D) or to omit the chicken serum from the maintenance solution (protocol E). As a result of following protocol D, numerous intracytoplasmic inclusions formed (Figs. 17 and 18); fewer inclusions were seen in the cultures from protocol E. Since the rate of destruction of cells was less at 30° C. than at 36° C., many more healthy, polygonal cells persisted for a period of 4 days in cultures kept at 30° C. than at 36° C. It appeared as though destruction of cells occurred too rapidly at 36° C. to permit extensive formation of inclusion bodies; whereas, at 30° C., cells remained intact sufficiently long to permit the formation and persistence of inclusions.

SUMMARY

Cells from a stable strain of human epithelium (HeLa, Gey) kept under continuous cultivation *in vitro* since its derivation from an epidermoid carcinoma of the cervix in February, 1951, were found to support multiplication of the viruses of herpes simplex, pseudorabies, and vaccinia. Each virus caused destruction of the cells and produced either intranuclear inclusion bodies (herpes simplex and pseudorabies) or intracytoplasmic inclusion bodies (vaccinia). The destructive effects of herpes simplex or vaccinia virus were seen usually within 1 to 2 days after inoculation of virus, whereas for pseudorabies virus the cytologic changes were commonly delayed for at least 3 to 4 days. Pseudorabies virus frequently caused discrete focal areas of cellular destruction; with herpes simplex or vaccinia virus, foci were seen only when a small viral inoculum had been employed.

We sincerely appreciate the assistance of Mrs. Alicia Hoogasian who contributed greatly to the studies reported in this series of papers.

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[Illustrations follow]

LEGENDS FOR FIGURES

Unstained preparations of cells are shown in all figures except Figures 6, 7, 13, 17, and 18; for the latter, cells were fixed in Bouin's solution and stained with hematoxylin and phloxine. All photographs of stained cells were made by Mr. Henry Morris.

- FIG. 1. Strain HeLa cells kept at 36° C. for 1 day in CHS-10, MS-90, and photographed immediately before the inoculation of virus. $\times 150$.
- FIG. 2. Cells 1 day after inoculation of herpes simplex virus; early evidence for cellular infection is shown by the change from polygonal cells to single round cells and to agglomerate multinucleate clumps. $\times 150$.
- FIG. 3. HeLa cells 2 days after inoculation of herpes simplex virus. Many of the cells are round, clumped, and present distinct nuclear borders. $\times 150$.
- FIG. 4. Strain HeLa cells in a control uninoculated culture photographed on the same day as the cells in Figure 3. $\times 150$.
- FIG. 5. A focus of destruction in a tube culture of strain HeLa cells, 3 days after infection by herpes simplex virus. $\times 75$.
- FIG. 6. Large inclusion bodies are contained in the nuclei of a multinucleated cell. $\times 800$.
- FIG. 7. Type A intranuclear inclusions in strain HeLa cells are present 2 days after infection with herpes simplex virus. $\times 800$.

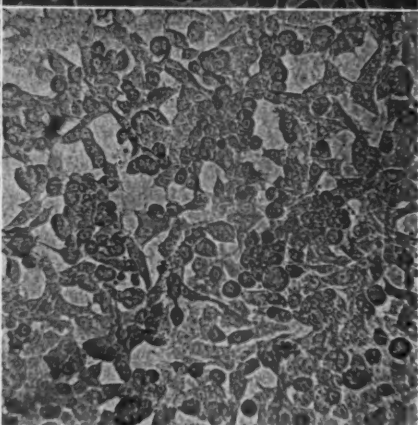
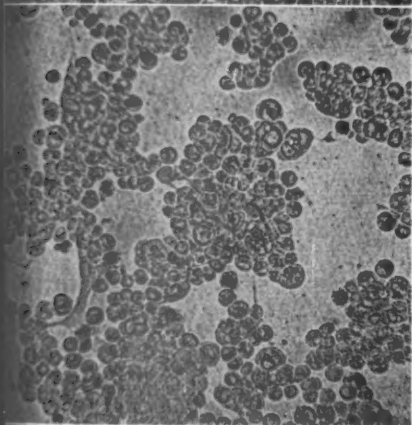
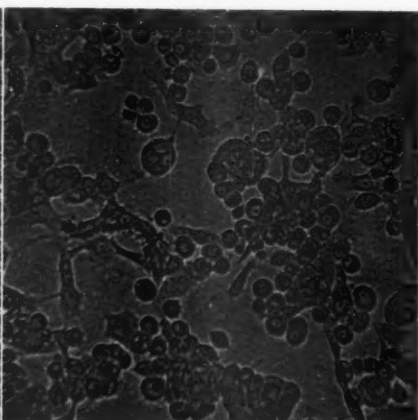
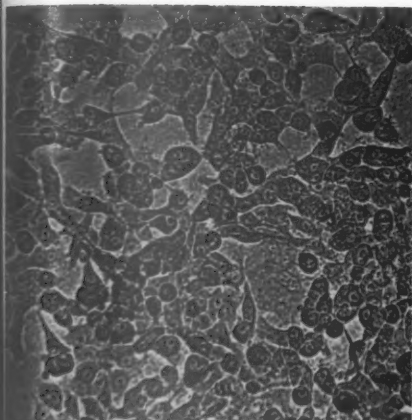
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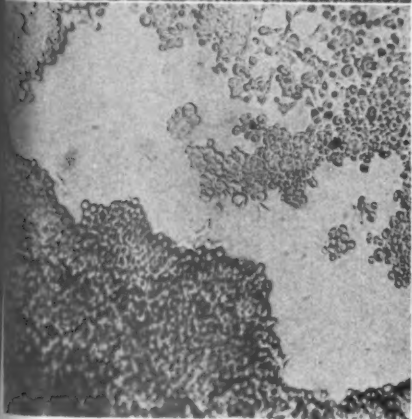
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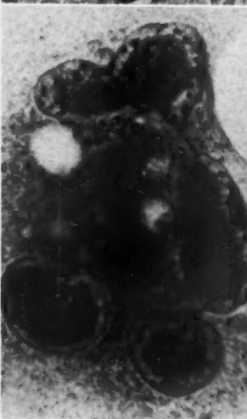
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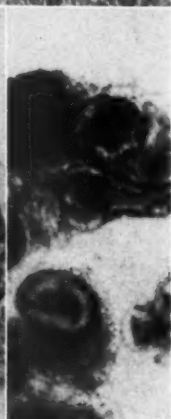
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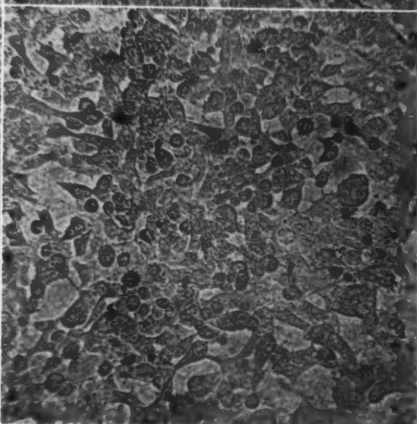
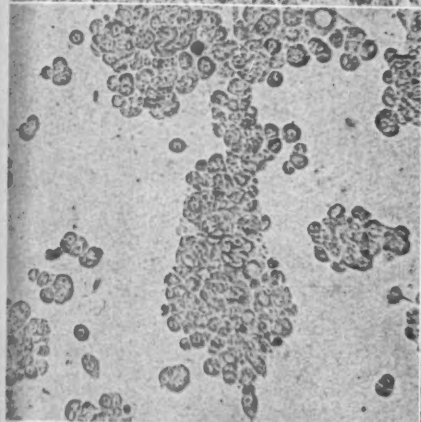
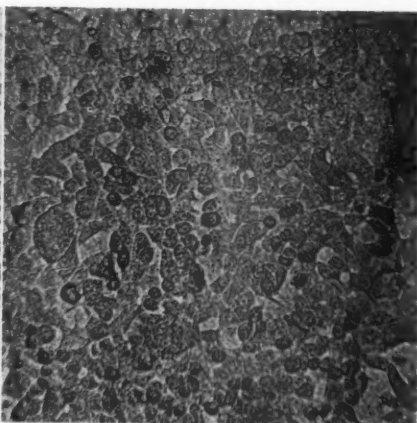
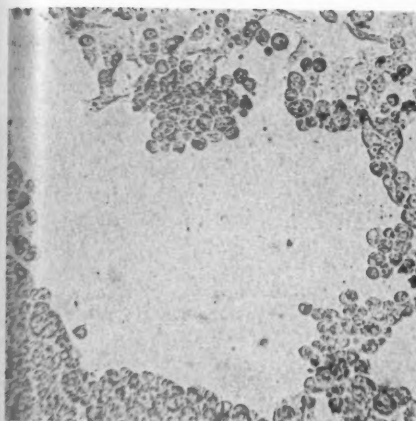
- FIG. 8. Effect of pseudorabies viral infection of strain HeLa cells, photographed 3 days after inoculation of virus; a focal area of cellular destruction is surrounded by a zone of round, degenerate cells and, beyond, by a zone of normal appearing cells. $\times 150$.
- FIG. 9. Normal appearing strain HeLa cells in an uninoculated control culture photographed concomitantly with the culture shown in Figure 8. $\times 150$.
- FIG. 10. Round, clumped, degenerate strain HeLa cells, 4 days after inoculation of pseudorabies virus. $\times 150$.
- FIG. 11. Normal cells in a culture without virus photographed for control purposes on the same day as the culture shown in Figure 10. $\times 150$.
- FIG. 12. Macroscopically visible foci of destruction from infection by pseudorabies virus (clear acellular areas in the sheet of strain HeLa cells on the glass surface of the test tube).
- FIG. 13. An intranuclear inclusion body of Type A in a cell infected by pseudorabies virus; inclusions were found commonly near foci of degeneration like that shown in Figure 12. $\times 800$.

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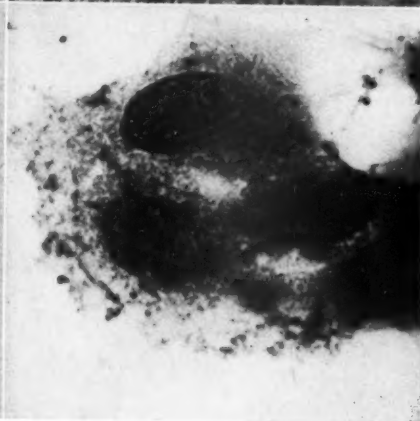
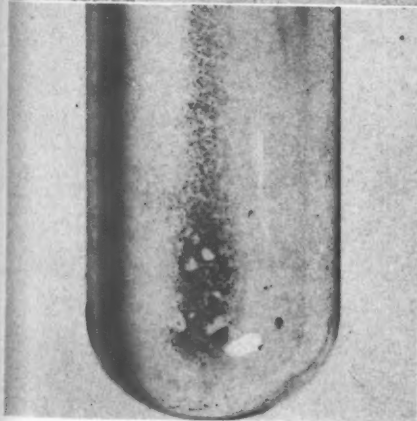
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FIG. 14. Strain HeLa cells infected by vaccinia virus, 1 day after the addition of vaccinia virus; evidence for infection is shown by the degenerate and round cells. $\times 150$.

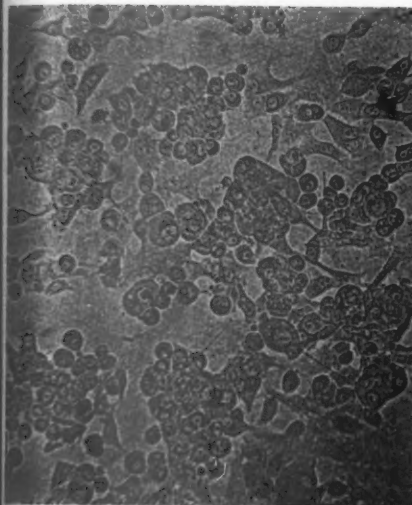
FIG. 15. Extensive cellular destruction that resulted 2 days after infection of strain HeLa cells by vaccinia virus. $\times 150$.

FIG. 16. On the fourth day after inoculation of vaccinia virus, only a few round, degenerate cells remained on the glass. $\times 150$.

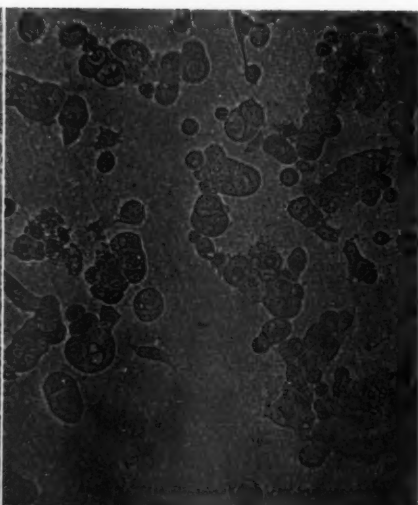
FIGS. 17 and 18. Guarnieri bodies in strain HeLa cells; after 2 days at 30° C. $\times 800$.

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THE VIRAL RANGE IN VITRO OF A MALIGNANT HUMAN EPITHELIAL CELL (STRAIN HELA, GEY)

II. STUDIES WITH ENCEPHALITIS VIRUSES OF THE EASTERN, WESTERN, WEST NILE, ST. LOUIS, AND JAPANESE B TYPES *

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The successful propagation and cytopathogenic effects of the viruses of poliomyelitis, herpes simplex, pseudorabies, and vaccinia in cultures of human malignant epithelial cells cultivated *in vitro* (strain HeLa, Gey) have been reported.¹⁻⁴ The purpose of this article is to present information that relates to the destructive effects and multiplication in cultures of strain HeLa cells, of the arthropodal-borne encephalitis viruses, of Eastern equine encephalomyelitis (EEE), Western equine encephalomyelitis (WEE), West Nile, St. Louis encephalitis (SLE), and Japanese B encephalitis (JBE).

MATERIALS AND METHODS

Viruses. The strain of EEE virus employed for these studies was isolated from a patient in 1951 by Dr. H. E. Dascomb, Louisiana State University. Infected mouse brain therefrom was passed intracerebrally once in mice in our laboratory for the preparation of a 5 per cent brain suspension in sterile 5 per cent dextrose in water. This brain suspension had an LD₅₀ of 10^{-7.5}/0.03 to 0.05 ml.

WEE virus and the Hubbard strain of SLE virus were obtained from the State of Minnesota Public Health Laboratory as 10 per cent suspensions of infected mouse brain. A single passage of WEE virus intracerebrally in mice yielded a 10 per cent suspension of brain tissue.

Lyophilized West Nile virus, labeled "25th passage, 10% MB, J 725960 in NMS, April 27, 1942 IHD lab.," available by courtesy of Dr. K. C. Smithburn, was purchased from the American Type Culture Collection. One passage intracerebrally in mice was carried out to make a 10 per cent suspension of infected brain.

A sample of JBE virus, Nakayama strain, as frozen and dried mouse brain, "5-17-48," was supplied by courtesy of Dr. C. M. Eklund. The virus employed was contained in 10 per cent suspension from the second mouse passage in our laboratory.

Viruses were stored in sealed ampoules at -70° C.

The methods for *viral assay*, *cellular cultivation*, *viral propagation*, and *photography* were described in the preceding article of this series.⁴

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Part I of these Studies is the immediately preceding article. Part III will appear in the January-February issue and will include a general discussion of the group. Part I should be consulted for explanation of certain abbreviations.—*Editor.*

† John and Mary R. Markle Scholar in Medical Science.

EXPERIMENTAL RESULTS

The purposes of these experiments were to learn (a) whether the virus under study would affect the morphologic characteristics of strain HeLa cells and (b) whether the virus would propagate. To accomplish these objectives, serial passage of virus was carried out in test tube or Porter flask cultures and microscopic observations of cells were made daily. The presence of virus in a culture was established by intracerebral inoculation of cultural liquid into mice and/or by the occurrence of cytologic changes in strain HeLa cells.

Eastern Equine Encephalomyelitis Virus

Cytologic Effects of EEE Virus. The destructive effects of EEE virus for strain HeLa cells became evident during the first passage of virus. To permit recording of these cytologic changes photographically, one passage was performed in Porter flask cultures.

Experiment 1. Two Porter flask cultures were prepared for viral inoculation by washing the cells and glass surfaces twice with MS-100, 1.0 ml. Chicken serum, 10 per cent, and maintenance solution, 90 per cent (CHS-10, MS-90), 1.0 ml., and EEE virus, 0.1 ml., from the 12th passage of experiment 2, were added to each flask. The fluid was removed from each flask before cells were photographed. It was replaced after photography when further incubation was desired. Photographs of cells from this experiment are shown in Figures 1 to 4.

The appearance of strain HeLa cells from a culture infected by EEE virus (Figs. 2 and 4) is in striking contrast to the appearance of cells in uninfected cultures (Figs. 1 and 3). This virus rapidly destroyed strain HeLa cells with the result, within 48 hours, of the disappearance of most cells from the glass (Fig. 2). However, a few cells often were spared and retained their normal shape for several additional days (Fig. 4).

Multiplication of EEE Virus. Serial passage of EEE virus in cultures of strain HeLa (experiment 2) was attempted to learn whether the virus would propagate.

Experiment 2. EEE virus, 0.05 ml., as mouse brain suspension, was inoculated into each of two test tube cultures of strain HeLa cells, containing 0.5 ml. of maintenance solution, 100 per cent (MS-100).^{*} The cultures for this experiment were not rinsed with balanced salt solution since antibodies for EEE virus were assumed not to exist in the human serum employed for cellular cultivation. The

^{*} Maintenance solution (MS-100) without chicken or other serum, 10 per cent, maintains strain HeLa cells well for at least 3 to 4 days at 37° C., if the old serum-containing medium used during the period of cellular cultivation is not removed completely by rinsing the cells and cultural vessel with salt solution. MS is enriched sufficiently to permit cellular maintenance without non-specific degeneration by the small amount of serum medium that remains in the vessel when the supernatant liquid is merely replaced.

medium containing serum was merely removed and replaced with MS-100. When degeneration of cells occurred, the supernatant liquids from two tubes were pooled, and aliquots, 0.05 ml., were transferred to each of two uninfected cultures to effect serial passage of virus. The data from experiment 2 are given in Table I.

The results of experiment 2 (Table I) show that EEE virus multiplied in cultures of strain HeLa cells. Virus infectious for mice and destructive for strain HeLa cells persisted through 20 serial passages,

TABLE I
Propagation in Vitro of Eastern Equine Encephalomyelitis Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture	Cumulative log of dilution of original viral inoculum	Results as indicated by		
			Cytopathogenic effect	Infectivity log of cultural liquid for	
Inoculum				Mice*	HeLa cells†
1	2	1.0	2/2‡	7.5	
5	12	5.0	3/3		
10	22	10.0	3/3	5.0	4.0-5.0
15	35	15.0	2/2		
20	48	20.0	3/3	> 3.0	5.0-6.0

* The results of mouse titrations in all tables are expressed as the negative log of the LD₅₀/0.05 ml. of diluted cultural liquid.

† The results of strain HeLa titrations are given as the negative log of the dilution of cultural liquid which, per 0.4 ml., produced a specific viral cytopathogenic effect, in a tube culture of cells, after 4 to 7 days of incubation at 36° C.

‡ For each of the tables, the numerator signifies the number of cultures that showed a viral cytopathogenic effect or the number of mice that died from viral infection. The denominator indicates the number of cultures or mice inoculated with virus.

over 48 days. The original virus inoculum (LD₅₀, 10^{-7.5}) was thus diluted 10²⁰ times. The results of titrations in strain HeLa cultures and in mice at the 10th and 20th passages add evidence that virus multiplied.

Western Equine Encephalomyelitis Virus

Cytologic Effects of WEE Virus. A cytopathogenic effect for WEE virus was uniformly obtained by the use of mouse brain suspensions although virus from several subsequent passages (experiment 4) failed to destroy strain HeLa cells. To learn whether this cellular destruction was specifically related to virus, neutralization with specific antibody was attempted (experiment 3). For photographic purposes, Porter flasks were used.

Experiment 3. Porter flask cultures were prepared for viral inoculation by washing with balanced salt solution and by the addition of CHS-10, MS-90, 1.0 ml. as in experiment 1. WEE virus, 0.1 ml., as a 2 per cent suspension of infected

mouse brain was added to each of four flasks. Human serum containing antibodies for WEE virus, kindly supplied by the Minnesota State Public Health Laboratories, was added to two cultures as 0.1 ml. of undiluted serum. Photographs that present the results of this experiment are shown in Figures 5 and 6.

A cytopathogenic effect of WEE virus for strain HeLa cells was seen 1 day after addition of virus (Fig. 5). In contrast to the nearly complete destruction of cells by WEE virus (Fig. 5), was the normal appearance of cells in the culture inoculated with specific antibody and virus (Fig. 6). The prevention of cellular destruction by the use of specific antibody established a causal relationship between WEE virus and its cytopathogenic effect for strain HeLa cells.

Multiplication of WEE Virus. The purpose of experiment 4 was to learn whether WEE virus would grow in cultures of strain HeLa cells.

Experiment 4. Serial passage of WEE was carried out in a manner similar to that for EEE virus (experiment 2). However, the cultures in this experiment were prepared for virus inoculation by washing the cells and walls of the cultural vessels twice with MS-100, or Hanks's salt solution, 0.5 ml., before the addition of CHS-10, MS-90, 0.5 ml. Virus was passed when destruction of cells occurred, or at approximately weekly intervals. The viral inoculum for the first passage was 0.05 ml. of 10 per cent suspension of infected mouse brains. The results of this experiment are given in Table II.

Data related to the propagation of WEE virus in cultures of strain HeLa are shown in Table II. Ten serial passages of virus, extending

TABLE II
Propagation in Vitro of Western Equine Encephalomyelitis Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture	Cumulative log of dilution of original viral inoculum	Results as indicated by		
			Cytopathogenic effect	Infectivity for mice	Titration in mice
1	2	1.0	2/2		
2	5	2.0	2/2	5/5	4.7
4	23	4.0	0/2	5/5	4.5
9	45	9.0	2/2	5/5	
10	50	10.0	2/2	5/5	

over a 50-day period, resulted in a 10^{10} dilution of the original viral inoculum. Fluids from passages 2 to 6, 9, and 10 were tested in mice and produced neurologic disease and death. Destruction of strain HeLa cells was seen in passages 1, 2, 5, and 7 to 10. In passages 3, 4, and 6, no cellular destruction occurred even though the cultures were incubated for longer periods than were used for other passages (11, 7, and 8 days, respectively). A reason for these failures of the virus to cause cellular damage has as yet not been learned.

West Nile Virus

Cytologic Effects of West Nile Virus. The cytopathogenic effect of West Nile virus for strain HeLa cells was seen in the first passage of virus. These cellular changes were recorded photographically in Porter flask cultures (experiment 5).

Experiment 5. The procedure for this experiment was similar to that for experiment 3 with WEE virus. The inoculum was a 10 per cent suspension of infected brain tissue from the first mouse passage of lyophilized virus. The cellular changes are shown in Figures 7 and 8.

West Nile virus destroyed strain HeLa cells (Figs. 7 and 8) though this cytopathogenic effect was usually delayed for at least 5 or 6 days after virus inoculation.

Multiplication of West Nile Virus. The ability of strain HeLa cells to support propagation of West Nile virus was tested in two experiments. For one (experiment 6), reconstituted lyophilized virus purchased from the American Type Culture Collection was used to inoculate the first passage cultures; for the other (experiment 7), a 10 per cent suspension of infected mouse brains from the first mouse passage of lyophilized virus was employed.

Experiments 6 and 7. The procedures for experiments 6 and 7 were similar to that employed for experiment 4 with WEE virus. The approximate dilutions of human serum medium effected by washing the cultures were $10^{3.3}$ to $10^{3.6}$ for passages 1 to 3; $10^{3.8}$ for passage 4 of each experiment; $10^{4.6}$ and $10^{5.6}$ for passage 5 of experiments 6 and 7, respectively; $10^{5.2}$ and $10^{6.2}$ for passage 6, and $10^{4.9}$ to $10^{5.2}$ for passages 7 to 12. The results of experiments 6 and 7 are given in Table III.

Evidence for the multiplication of West Nile virus in strain HeLa cells is shown in Table III. Virus was carried through twelve serial

TABLE III
Propagation in Vitro of West Nile Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture		Cumulative log of dilution of original viral inoculum	Results as indicated by					
				Cytopathogenic effect		Infectivity for mice		Titration in mice	
	Exp. 6	Exp. 7		Exp. 6	Exp. 7	Exp. 6	Exp. 7	Exp. 6	Exp. 7
1	6	5	1.0	2/2	2/2				
3	24	18	3.0	1/2	2/2	5/5	5/5	2.4	3.9
5	36	29	5.0	0/2	0/2	5/5	5/5		3.5
10	72	64	10.0	2/2	2/2	6/6	6/6	1.6	1.6
12	100	95	12.0	2/2	2/2	5/5	5/5		

passages in experiments 6 and 7, over periods of 100 and 95 days. The virus was uniformly infectious for mice. Destructive effects of West

Nile virus for strain HeLa cells were observed in all passages except 4, 5, and 6 in each experiment. Since passages 4, 5, and 6 in both experiments were carried out simultaneously, a common factor may have caused the absence of cytopathogenic effects.

St. Louis Encephalitis Virus

Cytopathogenic effects of SLE virus for strain HeLa cells were observed infrequently and irregularly. Yet, the virus multiplied.

Multiplication of SLE Virus. Serial passage of SLE virus was attempted in cultures of strain HeLa to determine whether the virus would multiply.

Experiment 8. The procedure for this experiment was similar to that employed for experiment 4 with WEE virus. The cultures for the first seven passages of this virus were washed to result in approximately a $10^{3.3}$ dilution of the human serum used in the medium for cellular cultivation; for passages 8 and 9 an approximate dilution of $10^{5.2}$ was effected. The inoculum for the first passage cultures was 0.05 ml. of 10 per cent suspension of infected mouse brain. The data from this experiment are given in Table IV.

Table IV presents evidence to show that SLE virus propagates in cultures of strain HeLa cells. Nine serial passages of virus, extending over a 72-day period, were carried out successfully. The pooled cultural

TABLE IV
Propagation in Vitro of St. Louis Encephalitis Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture	Cumulative log of dilution of original viral inoculum	Results as indicated by		
			Cytopathogenic effect	Infectivity for mice	Titration in mice
1	6	1.0	2/2		
2	25	2.0	0/2	5/5	2.4
5	44	5.0	0/2	5/5	3.6
9	72	9.0	0/2	6/6	

liquids from each passage produced in mice signs of encephalitis and death, 4 or 5 days after intracerebral inoculation. Confirmatory evidence for propagation of SLE virus was obtained from the results of titrations of fluid from the second and fifth passages.

SLE virus commonly did not exert a cytopathogenic effect for strain HeLa cells; destruction of cells was observed only in passages 1, 6, and 7. In other passage cultures, the cells retained a normal appearance for periods of from 6 to 19 days, despite the presence of virus as shown by mouse inoculation.

Japanese B Encephalitis Virus

Serial passage of JBE virus in cultures of strain HeLa cells was attempted to learn whether these cells were capable of propagating virus and/or of responding to infection by cytologic changes.

Experiment 9. Serial passage was initiated with JBE virus in 10 per cent mouse brain tissue, 0.1 ml., as the inoculum for each of two rinsed cultures of HeLa cells containing 0.9 ml. of CHS-10, MS-90. For each successive transfer at intervals not greater than 7 days, or when cytologic changes were observed, the supernatant fluids of the two cultures were removed, pooled, and an aliquot, 0.1 ml., was transferred to each of two tubes of HeLa cells. The data of this passage series are given in Table V.

TABLE V
Propagation in Vitro of Japanese B Encephalitis Virus in Cultures of Human Malignant Epithelial Cells (Strain HeLa, Gey)

Number of virus passage	Total days in culture	Cumulative log of dilution of original viral inoculum	Results as indicated by		
			Cytopathogenic effect	Infectivity for mice	Titration in mice
Inoculum				5/5	
1	5	1.0	2/2	1/4	
2	9	2.0	14/14	Not tested	
4	21	4.0	2/2	Not tested	
5	27	5.0	0/2	0/4	
10	61	10.0	0/2	5/5	2.3
13	66	13.0	0/2	6/6	

The data in Table V show that virus persisted through 13 passages over a period of 66 days resulting in dilution for the initial virus inoculum of a cumulative log of 13 or more. Overt evidence for cytopathogenic effect was present for 4 passages in the total destruction of all cells in from 4 to 6 days. During passages 5 to 13, most of the cells retained their normal structure; the attrition that results from such factors as age, nutrition, and low pH was held responsible for the degenerative and dead cells in cultures.

SUMMARY

Human epithelial cells (strain HeLa, Gey), cultured *in vitro* since their derivation from an epidermoid carcinoma of the cervix in February, 1951, were found to support the multiplication of encephalitis viruses of the Eastern, Western, West Nile, St. Louis, and Japanese B types. Eastern, Western, and West Nile types of virus regularly produced specific destruction of strain HeLa cells. It was shown with Western equine encephalomyelitis virus that cultures of strain HeLa

cells can be utilized for the demonstration of specific antibodies for the cytopathogenic encephalitis viruses. Cultures of strain HeLa cells were employed for titration of Eastern equine encephalomyelitis virus.

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LEGENDS FOR FIGURES

All photographs show unstained cells.

- FIG. 1. Strain HeLa cells kept at 36° C. for 1 day in CHS-10, MS-90, and photographed immediately before inoculation of virus. $\times 150$.
- FIG. 2. Strain HeLa cells, photographed 2 days after viral inoculation to show the destructive effects of Eastern equine encephalomyelitis virus. $\times 150$.
- FIG. 3. Normal strain HeLa cells in an uninoculated control culture, photographed on the same day as the culture shown in Figure 2. $\times 150$.
- FIG. 4. Strain HeLa cells, photographed 4 days after inoculation of Eastern equine encephalomyelitis virus to demonstrate that a few normal appearing cells remain, although extensive destruction has occurred. $\times 150$.
- FIG. 5. The cytologic effect of Western equine encephalomyelitis virus for strain HeLa cells as seen 1 day after the inoculation of virus. $\times 150$.
- FIG. 6. Normal appearing strain HeLa cells protected by specific antibody from the cytopathogenic effect of Western equine encephalomyelitis virus. $\times 150$.
- FIGS. 7 and 8. Destruction of strain HeLa cells by West Nile virus, photographed 6 days after the inoculation of virus. $\times 125$.

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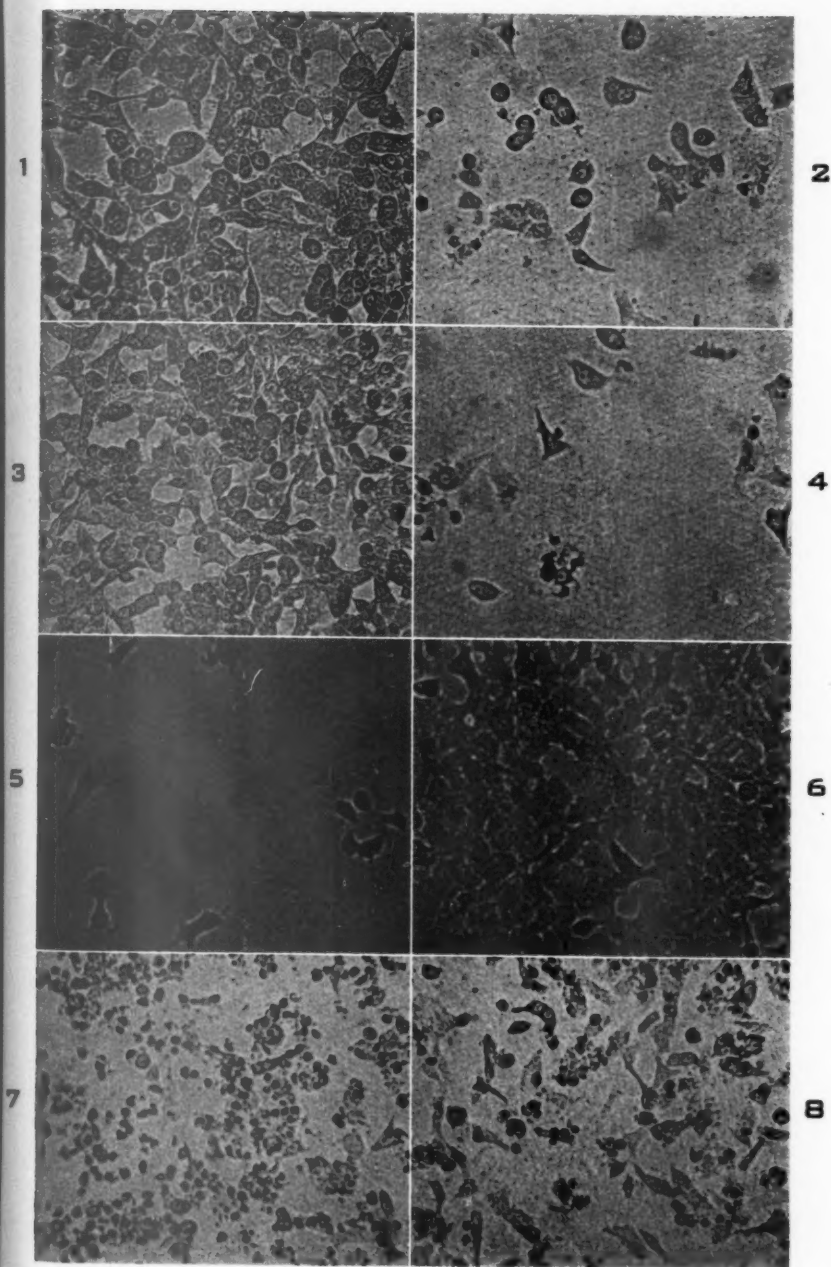
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AN EXPERIMENTAL STUDY OF THE VENOUS COLLATERAL CIRCULATION OF THE LUNG

I. ANATOMICAL OBSERVATIONS *

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Evidence has accrued recently of the existence of an extensive venous collateral circulation in some types of pulmonary disease in man, especially in emphysema.^{1,2} Quantitative estimates of the volume of the blood flow through these collaterals are difficult, if not impossible, to obtain in human subjects with methods currently available. This fact has prompted an attempt at the experimental production and functional evaluation of such a collateral circulation in the dog. The general pattern established in an earlier experimental investigation of bronchial arterial collateral circulation has been followed.³

Although dogs could survive ligation of the venous drainage of a pulmonary lobe, it was long thought that this procedure was inevitably followed by hemorrhage, "atelectasis," and often by pneumonitis and thrombosis of the pulmonary veins.⁴⁻⁹ The clinical observation that tuberculosis is rarely progressive in lungs which are the seat of chronic passive congestion, received experimental support from Tiegel,⁵ who constricted the pulmonary veins in dogs and rabbits. A similar operation has since been used in the treatment of pulmonary tuberculosis in man.⁷ When penicillin came to be employed postoperatively, it was found possible to reduce strikingly the mortality following ligation of the veins of an entire lung.^{10,11}

Although gross and histologic changes after this operation have been described in detail, especially by Wyatt and associates,¹¹ there is little information regarding the collateral circulation. Schlaepfer¹² demonstrated both hilar and pleural collateral vessels in the dog after simply constricting lobar veins to one half or one third of their original caliber. The state of the pulmonary veins after this procedure was not described, although it would be of interest to know whether they remained patent.

In the present investigation the main efforts were to determine the relative effective flow through the collateral veins, to demonstrate their

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courses and relationships to the pulmonary vessels, and to consider the mechanisms governing their development. The functional observations will be reported separately.

MATERIALS AND METHODS

The pulmonary veins were ligated extrapericardially on the right side in 4 dogs and on the left in 6. Except in the first 2 animals in which only the veins in the upper lobe were ligated, the attempt was made to interrupt the continuity of all of the pulmonary veins of one lung. That the drainage of the right mediastinal lobe may cross the midline to join that of the left lower lobe must be considered in the operative procedure. Occasionally, portions of lobes may be drained by separate small veins directly into the atrium, although the prevailing pattern necessitated the ligation of three major veins at their entrance into the atrium on the left side, and from three to five for the right lung. A most important step in the care of the animals was the administration of antibiotics. In the present work both penicillin (200,000 units) and streptomycin (0.5 gm.) were administered intramuscularly for 5 days postoperatively.

After bronchspirometry and catheterization studies (Fig. 1) had been completed, each animal was heparinized and sacrificed with an overdose of pentothal for necropsy and the preparation of a vinylite cast of the tracheobronchial tree and vascular structures. The general methods for making such casts have been described elsewhere¹³ as have the special procedures in producing a cast of the azygos system.² Certain additional modifications were found useful for present purposes. The superior vena cava was transected just above the azygos vein and the distal end cannulated. A cannula was introduced into the mouth of the azygos vein, and this vessel was ligated just above the diaphragm. The azygos vein was perfused successively with water, air, acetone, and 10 per cent yellow vinylite. Each of these materials could be seen to issue from the cannulated superior vena cava, indicating connections between the azygos and caval systems. As the vinylite appeared, the caval cannula was occluded in order to permit distention of the azygos venous system with vinylite, and thick (28 per cent) yellow vinylite was then introduced into the azygos vein, and, in the opposite direction, into the superior vena cava, while the azygos cannula was kept closed. The two cannulas were then joined by means of a Y-tube and appropriate connections of rubber tubing, and reinjection of the systems accomplished during several days to compensate for shrinkage of the vinylite. The aorta was injected *in situ* with black

plastic. Following this procedure, the entire thorax and its contents were freed by transecting the spinal column and soft tissues in the neck and in the abdomen below the last rib. The isolated thorax was suspended in a vacuum jar after cannulating the trachea and vascular structures, which were then injected as described in the study of the bronchopulmonary venous circulation in man.² White plastic was used for the tracheobronchial tree, red for the pulmonary arteries, green for the pulmonary veins, yellow for the azygos and superior vena cava, and black for the aorta.

OBSERVATIONS

Inspection *in situ* revealed that the lung at the site of ligature was usually firmly adherent to both the mediastinum and the lateral wall of the chest, in and close to the line of incision. In 2 animals, however, adhesions were confined to the mediastinal aspect of the pleura. The adhesions were in all instances remarkable for their vascularity. In addition, large thin-walled veins could be seen to traverse the loose connective tissue anterior to the pericardial sac from the internal mammary vein to the adherent pleura and to disappear finally into the pulmonary substance (Fig. 2).

Visual inspection of the fresh tissues yielded only the most rudimentary impression of the extent of the collateral circulation that was demonstrated in the casts. Even during the preliminary injection of air into the cannulated azygos vein it became apparent that freer communications than usual existed between the tributaries of this vessel and the superior vena cava. The two were connected by contributing branches that served as collaterals to the same pulmonary veins, as well as through the spinovertebral system. In the casts prepared by digesting the entire thorax, this system was demonstrated with utmost clarity in its relationship to the azygos vein and vena cava in the manner previously described by Batson¹⁴ and others before him, as recounted by Harris.¹⁵ The azygos vein was connected to this system at each somatic segment by branches of an intercostal vein, and the major connections with the superior vena cava were through the superior intercostal vein.

It was apparent in the casts that most of the pulmonary veins had not suffered thrombosis, despite the ligature of the major vessels at the hilum 3 to 5 months previously, and that they remained in continuity with two main types of collateral channels. One set of collaterals, probably largely expanded pre-existing bronchial veins, joined the proximal ends of the major pulmonary veins at the hilum. The second set represented transpleural vessels that joined the hilar plexus just

described (transpleural hilar collaterals), or connected directly with the distal ends of small pulmonary veins (transpleural terminal collaterals). The anatomical capacity of these collateral vessels appeared compatible with the observed blood flows that approximated in some instances 20 per cent of that expected in the intact lung.

Expanded Bronchial Collateral Veins

Normally, the bronchial veins drain the first several orders of bronchi in the dog, and are connected with major pulmonary veins by means of very short but wide transverse channels. By the method employed, they were only rarely injected from the azygos or its branches in the normal dog, even with dilute (10 per cent) vinylite, probably in consequence of valves. They were occasionally injected from the pulmonary veins, however, with plastic of the same concentration, containing as much as 5 per cent suspended diatomaceous earth. The largest of these vessels encountered is illustrated in Figure 3. They were smaller and much less easily injected than in adult man.

After ligation of the pulmonary veins the bronchial veins were found to have expanded in a most remarkable fashion (Figs. 4 to 14). On the walls of bronchi of the proximal two or three orders these channels retained their plexiform arrangement and connected with the proximal end of the major pulmonary vein of the lobe (Fig. 4), or with a segmental vein, which was thus injected retrogradely and which displayed its characteristic fern-like distribution peripherally (Figs. 5 and 7). The connections of these bronchial veins with the pulmonary veins tended to be small but multiple (Figs. 4, 7, and 14). The hilar bronchial veins then entered the mediastinum, where they were joined by venules from the esophagus and other mediastinal structures, retaining for a time their net-like arrangement, but then becoming gathered into several major vessels that entered the intercostal veins or the azygos itself.

Detailed descriptions of the hilar collaterals are given in the Appendix. In general it may be said that the major patterns were similar, whether the right or the left major pulmonary veins had been interrupted. Hemiazygos veins were not encountered in the dog.

The major bronchial venous channels varied in number from two to four. In all casts at least one of these veins drained into the azygos directly and in all but one (dog V₃) there was an additional vein that drained into a homolateral intercostal, usually the common stem of the fourth and fifth intercostal veins.

The largest single bronchial vein, found in dog V₃, had a diameter

of 8 mm., eight times that of the largest found on the control side in any of eight casts, and the sum of the diameters of the major bronchial veins in this dog was 14 mm. This does not include the newly formed transpleural hilar collaterals or the veins as described later. If these vessels were included, the total cross sectional diameter of all the draining vessels in different animals varied from 11 to 18 mm. Even these measurements did not include the transpleural terminal collaterals. These measurements serve only to give an inkling of the relative size of these vessels, and no implication is intended regarding rates of flow or other functions on a quantitative basis.

Transpleural Hilar Veins

Hilar connections also became established between transpleural veins and the proximal ends of the pulmonary veins. The former were thus quite analogous to the bronchial veins, but subserved their function chiefly in the upper parts of the lungs that were less accessible to the azygos and its branches. These transpleural vessels were obviously newly formed, from capillaries of granulation tissue in the developing pleural adhesions. Such collaterals may reach a diameter of 4 mm. (Figs. 11 and 16). They were derived most commonly from the pericardiophrenic vein, and occasionally from a descending branch of the superior intercostal, or from the subclavian or brachial vein.

Transpleural Terminal Veins

The most remarkable of the collateral veins, present in all casts, were newly formed vessels that penetrated the pleura and joined the distal ends of pulmonary veins, end to end (Figs. 9 and 10). The sources of such transpleural terminal vessels were usually chiefly the pericardiophrenic and intercostal veins, but in several instances they were branches of internal mammary veins (Figs. 11 and 13). In several specimens there were also vessels from the subclavian, brachial, azygos, and diaphragmatic veins. The intercostal collaterals were largest and most numerous at the level of the surgical incision and one or two segments above and below. On the other hand, in two specimens, the lung was not adherent at all to the lateral wall of the thorax. In those instances other transpleural veins on the mediastinal aspect were relatively large (Fig. 9).

Completeness of Interruption of Venous Drainage

It was found that surgical interruption of the venous drainage had been complete only in the last 3 animals of the present series. This

fact readily became apparent when the open pulmonary veins were injected from the atrium. An appropriate correction in the observed results of the functional studies was therefore required in those five instances in which the ligation was incomplete. Some useful anatomical information nevertheless was contributed by the very fact that certain of the pulmonary veins had escaped ligation. Even in a sub-segment it was apparent that the only way in which the uninterrupted pulmonary veins were connected with those beyond the ligation was by the hilar network of bronchial veins (Fig. 11). Furthermore, there was no evidence that any transpleural collaterals had established connections with intact pulmonary veins, although some of the expanded hilar bronchial veins had been injected in green plastic via the pre-existing proximal connections with these vessels.

MICROSCOPIC OBSERVATIONS

Since the lungs were used primarily for the preparation of casts, detailed gross and microscopic observations of the pulmonary tissue cannot be presented. Such studies have been reported by Wyatt and his collaborators¹¹ on animals that had received penicillin. There is some evidence that less injury to the lung results from the operative procedure when both penicillin and streptomycin are administered postoperatively as in the present experiments. The incidence of sanguineous sputum was less. In the observations reported here the lungs appeared well aerated and were capable of considerable respiratory exchange, being thus more than the mere space-occupying organs suggested from the preliminary functional observations of Hanlon and his co-workers.¹⁰ In most instances they were but slightly, if at all, smaller than expected, but in several they were definitely small, perhaps partly in consequence of entrapment by unusually dense adhesions upon both the lateral and mediastinal aspects.

The lungs of only one animal (V5), which died 4 months after ligation of the veins while under anesthesia preparatory to the physiologic studies, were examined microscopically. Except for changes in the pleura, the lungs of the operated side were difficult to distinguish from the control (Figs. 15 and 16). On the side of ligation the alveoli in some zones seemed slightly thicker and more congested. This was, however, considered to be within the expected range of variation of the lung under the circumstances. There was no evidence of the focal fibrosis described by Wyatt¹¹ for lungs after 5 months to 1 year following venous occlusion. There was no thrombosis of the vessels. The microscopic study in dog V5 supplements our observations on the

patency of the vessels as seen in the casts. A minute polypoid intrusion of connective tissue was found in the lumen of one bronchiole, suggesting a pre-existing bronchitis, and several small foci were seen where intra-alveolar phagocytes were accumulated in small groups and were filled with hemosiderin pigment. Both of these findings may be explained on the basis of the passage of helminthic parasites through the lungs, especially since they were observed bilaterally. Such changes, moreover, are occasionally encountered in unoperated dogs.

DISCUSSION

A definite contribution to these experiments has been made by the antibiotics, penicillin and streptomycin. Under the protection of these drugs used together, the mortality from ligation of the major veins of a lung has been nil, in contrast with animals receiving penicillin alone as in the series of Wyatt and associates.¹¹ Only 2 of the 10 animals had a cough productive of blood-tinged mucus for several days, but quickly became asymptomatic. In contrast with some accounts of venous ligation in pre-antibiotic days, the ligated pulmonary veins did not become thrombosed. There were no significant histologic changes in the lungs of the one animal which were studied 4 months after ligation. This would suggest a contribution of infection to "infarction," that can be abolished under some circumstances. An analogy exists in the hepatic circulation of the dog in which Markowitz, Rappaport, and Scott¹⁶ have demonstrated that necrosis of the liver after ligation of the hepatic arteries can be prevented by the use of penicillin.

As the venous collaterals of the lung develop, there is an obviously broader and more direct access to the azygos-spinovertebral system. This may have some significance in chronic suppurative or neoplastic disease of the lung in which collateral venous drainage may be expanded.^{1,2}

It is interesting to note that expansion of the venous and arterial collateral circulation is not necessarily concomitant. There was no evidence of change in the latter during the course of the present experiments, nor was the venous side found to be expanded despite the extensive increase of the arterial collateral circulation that takes place after ligation of the pulmonary artery.¹⁷ In man the two circulations may or may not be simultaneously expanded in the course of disease.

The mechanisms governing development of the venous collateral circulation are doubtless complex and not entirely understood. They were certainly rapidly efficient within 3 months, as judged not only by the actual size of the venous channels but also by their capacity to

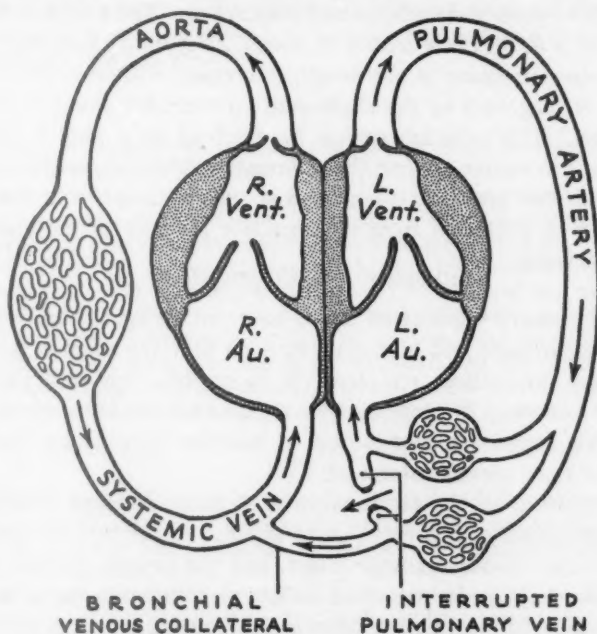
carry oxygenated blood from the lung. The expansion of pre-existing collaterals at the hilum is doubtless associated with an immediate increase in pressure within them at the moment of ligation of the pulmonary vein.¹⁸ It will be recalled that the bronchial veins are normally in free communication with the large pulmonary veins near the hilum through stomas of precapillary size. With the increased flow through these hilar bronchial veins there is an increase in their size—as in response to the same mechanical stimulus in other vessels.¹⁹ As in the developing embryo, those vessels that carry the greatest flow are destined to become the principal channels. A full explanation of why greater blood flow through a vessel results in an increase in its size is not as yet available.

Even more obscure are the forces that seem to guide the transpleural collaterals, especially in their direct end-to-end junction with the pulmonary veins. In reaching these last-mentioned collaterals the blood passes from the pulmonary artery to the alveolar capillaries, thence to the still patent pulmonary veins, and only then to the collaterals with which they are connected by openings whose size may reach 2 mm. Since transpleural collateral vessels must be newly formed from capillaries within the granulation tissue of adhesions, it is puzzling why the predominant drainage of the alveolar capillaries does not appear to be directly into these collaterals, but rather is mediated by the pulmonary veins. Evidence of the interposition of the latter are the observations that the collateral veins do not ramify far into the parenchyma and their branches are few, and that each terminal becomes connected at once with a pulmonary vein near the pleural surface and without decrement in size. The connections with the latter are created at a point where the pressure has fallen to its lowest level after the blood has entered the lung via the pulmonary arteries. Indeed it may even be asked why the venous collaterals never have been observed to form a short-circuit by connecting directly with the pulmonary arteries, as might seem reasonable if a mechanical force were the determining factor. The efficiency of the actual arrangement is obvious, since the pulmonary veins are already connected with the vast capillary bed of the lung, and, after ligation of the pulmonary veins, the simplest alternative drainage would be by establishing directly precapillary connections with these still patent vessels. The method by which this is accomplished is still obscure.

The converse has been observed also in the development of the arterial collateral circulation where the transpleural systemic collateral vessels are connected directly with the pulmonary *arteries*. This has

been observed after the experimental ligation of the main arterial supply to the lung in the dog, or in congenital pulmonic stenosis. In this instance we have never observed precapillary connections between the collateral arteries and the veins.

A possible application of the ligation of the pulmonary vein in the therapy of transposition of the great vessels suggests itself. It is obvious that in this congenital anomaly there are two independent circulations—a systemic through the right side of the heart and a lesser involving the left—except as some crossing over is effected through septal defects, patent ductus arteriosus, and possibly certain other shunts. Frequently in these instances, the ductus is closed and the septal defects are exceedingly small.^{20,21} If the drainage of one lung could be directed toward the right side of the heart (as by ligation of pulmonary veins), a volume of highly oxygenated blood would be added to the desaturated systemic blood (Text-Fig. 1). It is interest-



Text-fig. 1. This diagram indicates a possible application of ligation of the pulmonary vein of one lung in the therapy of transposition of the great vessels. The blood leaving this lung would be directed to the right heart, thereby contributing richly oxygenated blood to the systemic circulation. Possible pathways for the necessary return of an equivalent volume of blood to the lesser circulation are not indicated, but might consist of trans-septal or extracardiac shunts that can be expected to accommodate to the induced, gradually increasing, flow from left to right.

ing to note that although death occurs very early in most instances, in one patient with this congenital lesion who survived to the age of 38 (reported by Messeloff and Weaver²²), the pulmonary veins were congenitally transposed, draining into the right atrium. It is important, of course, that not only oxygenated blood be provided to the systemic circulation but also that some means exist to permit a return of blood to the pulmonary circulation. It is likely that available pathways for the latter function would become more efficient after the creation of the shunt to subserve the former. One special danger may exist in these patients consequent to the pulmonary arterial hypertension which is probably present in most of them. Evidence for this hypertension is the generally observed hypertrophy of the ventricle of origin of the pulmonary arteries and the thick wall and large diameter of the main pulmonary artery itself. It might then be that upon obstructing the pulmonary vein, the capillary pressure would be so increased as to lead to pulmonary edema and hemorrhage. The evidence for this possibility is in the observations of Shedd, Alley, and Lindskog.¹⁸ That the capillary pressure is not greatly increased with the veins unobstructed is suggested by the absence of an excessive venous collateral circulation.² This question cannot be resolved on a purely theoretic basis, since in course of time the pulmonary arterioles become altered by sclerosis and muscular overgrowth in such a manner that the capillaries may be protected from the excessive pressure in the main pulmonary arteries.

SUMMARY AND CONCLUSIONS

The pulmonary veins of an entire lung can be ligated without producing hemorrhage and necrosis, provided that the animals are protected with antibiotics; streptomycin in combination with penicillin appears to be more favorable in this regard than the latter alone. This observation suggests a contribution of infection to what has formerly been considered merely infarction.

After ligation of the pulmonary veins there develops a collateral circulation capable of carrying up to at least 20 per cent of the blood flow were the venous drainage intact, and the oxygen content of the azygos blood rises. In part, this collateral circulation depends upon the expansion of pre-existing bronchial venous channels, probably in response to mechanical factors, and, in part, upon the development of new channels of large size from the capillaries of granulation tissue within pleural adhesions. Some of these supplement the hilar bronchial veins, but others join the pulmonary veins, end to end, at the periphery. The factors guiding this junction are not known, but in their discovery may lie an important key to the mechanisms of collateral circulation in

general. The arterial collateral circulation is not altered concomitantly.

Since, in consequence of ligation of the pulmonary veins, expanded collateral veins of a lung can be caused to drain oxygenated blood via the azygos and innominate veins into the right heart, this operation suggests itself as a simple procedure possibly applicable to the partial correction of the disturbed hemodynamics of congenital transposition of the great vessels.

APPENDIX—DESCRIPTION OF CASTS

Dog V₃

Right lung. All veins were ligated except those of the mediastinal (cardiac) segment: ligation considered 33 per cent incomplete.

A. Bronchial Hilar Collaterals. 1, an 8 mm. trunk from the medial aspect of the azygos vein at level of the fourth intercostal space. 2 and 3, two 2 mm. trunks arising from the azygos at the level of the sixth intercostal space. 1, 2, and 3 join a plexus that drains most of the lower lobe.

B. Transpleural Collaterals. 4, branches from second, third, and fourth intercostal veins on lateral aspect of pleura. 5, pericardiophrenic branch draining chiefly the middle lobe. (See Figs. 3 to 8.)

Dog V₄

Right lung. Pulmonary vein to one third of the right middle lobe was not ligated: estimated to be 10 per cent incomplete.

A. Hilar Bronchial Collaterals. 1, a 5 mm. vein from the common stem of the fourth and fifth right intercostal veins. 2, a trunk, 2 to 3 mm. in diameter, from the posterior aspect of the azygos at the level of the third intercostal space.

B. Transpleural Collaterals. 3, a long branch, 1 to 2 mm. in diameter, descending to the left of the aortic arch from the left superior intercostal and then anteriorly to the aorta to join the upper branch of 1. 4, a pericardiophrenic branch from the superior vena cava, approximately 1 mm. in diameter (also yields transpleural branches). 5, a 4 to 5 mm. branch from the innominate vein that enters the upper lobe. 6, small branches from the left side of the superior vena cava. 7, diaphragmatic branches entering the anterior margin of the mediastinal lobe. (See Figs. 9 and 10.)

Dog V₆

Left lung. Two small pulmonary veins not ligated: a small lingular branch draining approximately one third of the posterior portion of this segment, and the main branch from the first dorsal bronchus of the lower lobe. Ligation estimated to be 25 per cent incomplete.

A. Bronchial Hilar Collaterals. 1, from left intercostal trunk common to fourth and fifth intercostals, just beyond its origin on the left lateral aspect of azygos at the level of fifth intercostal. 2, a trunk from the anterolateral aspect of azygos, 3 mm. below origin of sixth left intercostal vein. 3, small 1 to 2 mm. posterior descending branch from left superior intercostal.

B. Transpleural Collaterals. 4, transpleural branch (4 to 5 mm.) of internal mammary drains upper lobe.

There is evidence of possible cross circulation from a ligated to an unligated vessel. In the lingula and lower lobe, the former have been injected retrogradely with green plastic from the latter by way of a tortuous bronchial venous plexus. It is

probable, however, that the blood flow was from both sets of pulmonary veins into the collaterals. (See Fig. 11.)

Dog V7

Left lung. All venous drainage interrupted except for small branch in posterior portion of lingula. Ligation estimated to be 10 per cent incomplete.

A. Bronchial Hilar Collaterals. 1, a single huge trunk from the common source of the fifth and sixth intercostal veins on the left. 2, 3, and 4, from anterior aspect of azygos vein just below seventh, eighth, and ninth intercostal veins, respectively.

B. Transpleural Collaterals. 5, trunk, 4 mm. in diameter, from brachial vein (incomplete). 6, complex network from pericardiophrenic (3 mm.). 7, diaphragmatic branches (2 mm.). 8, minute branches from fourth, fifth, and sixth intercostals traversing adhesions. (See Fig. 12.)

Dog V8

Pulmonary venous drainage completely interrupted.

A. Bronchial Hilar Collaterals. 1, branch from left lateral surface of arch of the azygos vein as it loops over the right main bronchus. 2, a 2 to 3 mm. branch from anterior surface of common trunk of fourth and fifth intercostal veins at level of the latter. 3, a branch, 3 mm. in diameter, from anterior surface of azygos vein at level of sixth intercostal. 4, from anterior surface of azygos at level of sixth to seventh intercostal (diameter, 3 mm.).

B. Transpleural Collaterals. 5, a 1 to 2 mm. branch from the pericardiophrenic vein. 6 and 7, from the third and fourth intercostal veins.

Dog V9

Pulmonary venous drainage completely interrupted.

A. Bronchial Hilar Collaterals. 1, from left lateral surface of azygos at level of fourth intercostal (diameter, 5 mm.). 2, small vessels from fifth intercostal (1 mm.). 3, from anterior surface of azygos just below sixth intercostal (1 mm.). 4, contribution from posterior branch of pericardiophrenic.

B. Transpleural Collaterals. 5, branch of 2. 6, branch of 3. 7, large (4 mm.) pericardiophrenic vein extending along central part of medial surface of upper lobe, lingula, and lower lobe. 8, branch of internal mammary (3 mm.) supplying the anterior surface of upper lobe. 9, branches of fourth and fifth intercostal veins. (See Fig. 13.)

Dog V10

Pulmonary venous drainage completely interrupted.

A. Bronchial Hilar Collaterals. 1 and 2, small branches, each 2 mm., from the fourth and fifth left intercostals respectively, passing to the left of the aorta and thence to a hilar position. 3, from the paravertebral segment of the left fifth intercostal (2 mm.). 4, from the azygos just below sixth intercostal (3 mm.).

B. Transpleural Collaterals. 5, from a pericardiophrenic branch passing to the left of the aorta and thence to the hilar aspect of the upper lobe (1 to 2 mm.). 6, from a long, thin vein, 1 mm. in diameter, draining into the superior vena cava after passing to the right of the aorta with the same destination as 5. 7, twigs from 1, 2, and 3. 8, an anterior marginal branch to the upper lobe. 9, an anterior marginal branch to lower lobe. 10, anterior marginal branches ascending from diaphragmatic veins. 11, branches of the fourth, fifth, and sixth intercostal veins. (See Fig. 14.)

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LEGENDS FOR FIGURES

- FIG. 1. Roentgenogram during functional study (dog V₄). The bronchspirometric cannula is shown with the expanded end of the inner element within the left main bronchus. The catheter, following a posterior arched course, is in position with its tip in the azygos vein.
- FIG. 2. Thorax of dog V₅ at necropsy, 5 months after ligation of the pulmonary vein. Newly formed transpleural collaterals traverse adhesions between the right lung, especially the right upper lobe, and the regions drained by the internal mammary and pericardiophrenic veins.
- FIG. 3. View of posteromedial aspect of cast of left atrium and unoperated left lung of dog V₃ to demonstrate the largest normal bronchial vein (diameter, 1 mm.) in the present series of dogs, as injected from the pulmonary vein. The small vessel (arrow) is seen against the background of the lower lobe bronchus. The points of ligation of the major pulmonary veins of the right side are seen as bulbous projections from the atrium. The transected stump of the mediastinal lobar vein (which was not ligated) also is visible.

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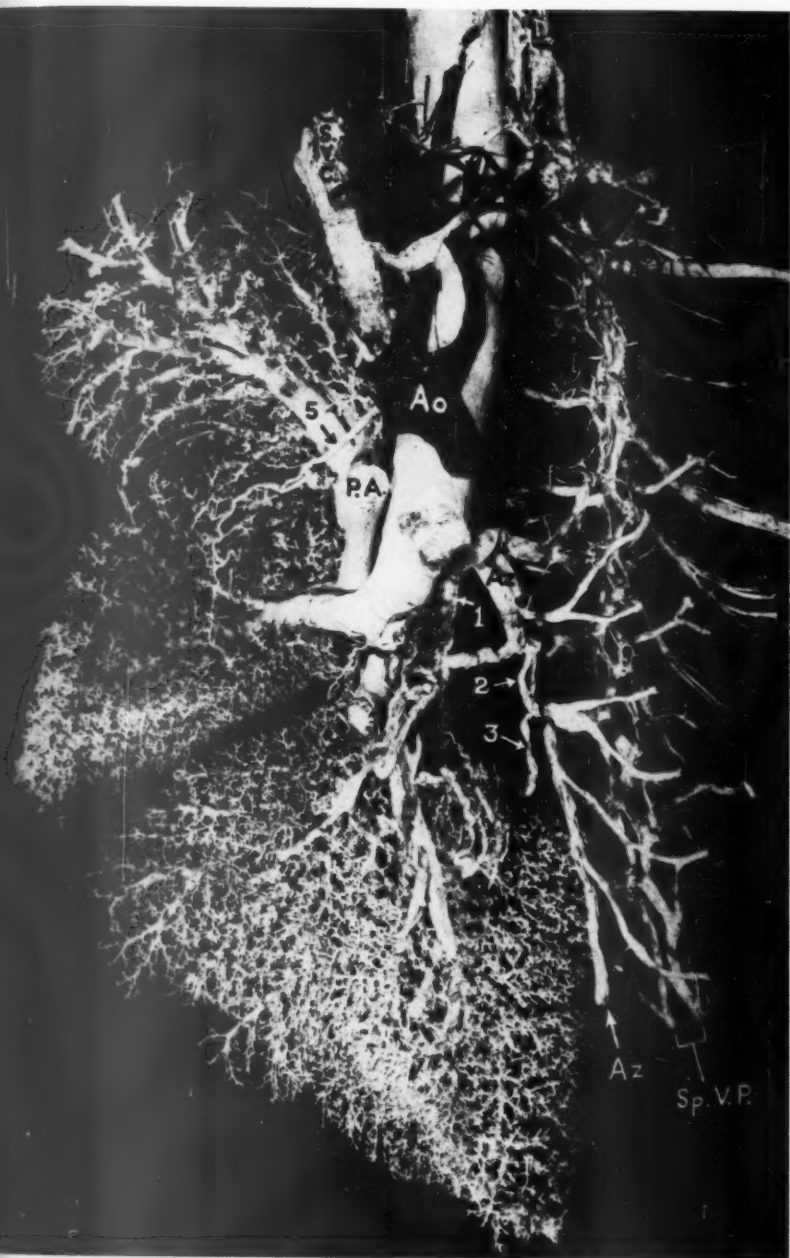
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- FIG. 4. Medial aspect of right lung of dog V3, 3 months after ligation of pulmonary veins. Bronchial hilar collateral plexus consisting of: 1. 8 mm. trunk arising from medial aspect of azygos vein at the level of the fourth intercostal space. 2 and 3. Branches of common trunk arising from azygos at level of the sixth intercostal space. Transpleural terminal collaterals to the middle lobe are seen arising from an enlarged branch of the pericardiophrenic vein, 5. Key: Az = Azygos vein. Sp.V.P. = Spinovertebral venous plexus. S.V.C. = Superior vena cava. P.A. = Pulmonary artery. Ao = Aorta.

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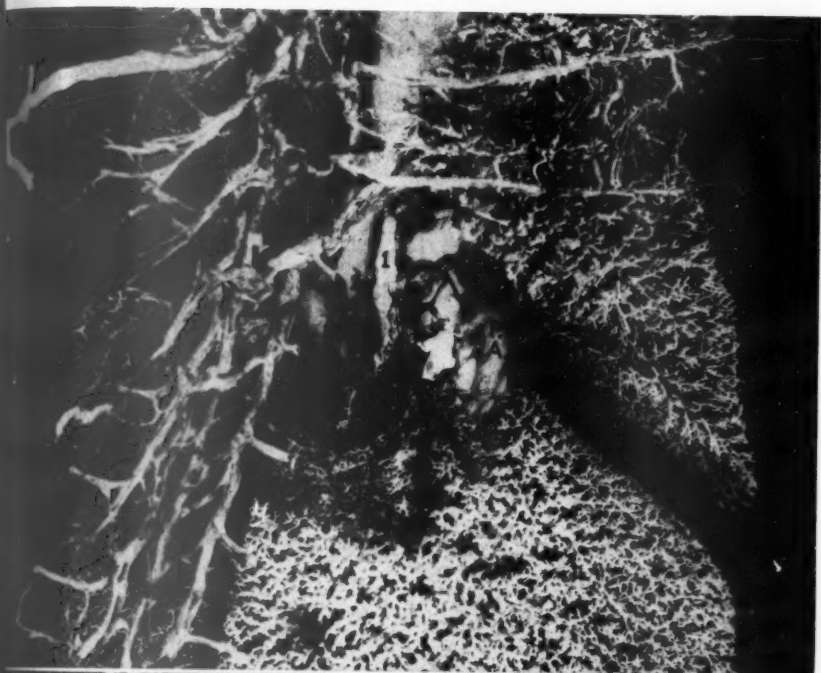
FIG. 5. Dog V3. Right and posterior aspect of cast. The enormously expanded hilar bronchial veins (labelled to correspond with Fig. 4), form a rete upon the major bronchi, and at one point can be seen to communicate with a segmental vein within the posterior portion of the right lower lobe that has been dissected to expose the vessels. (For comparison with Fig. 8.)

FIG. 6. Newly formed transpleural collateral terminal veins draining the right upper lobe into the third, fourth, and fifth intercostal veins. These collaterals are joined end-to-end with the distal extremities of small pulmonary veins.

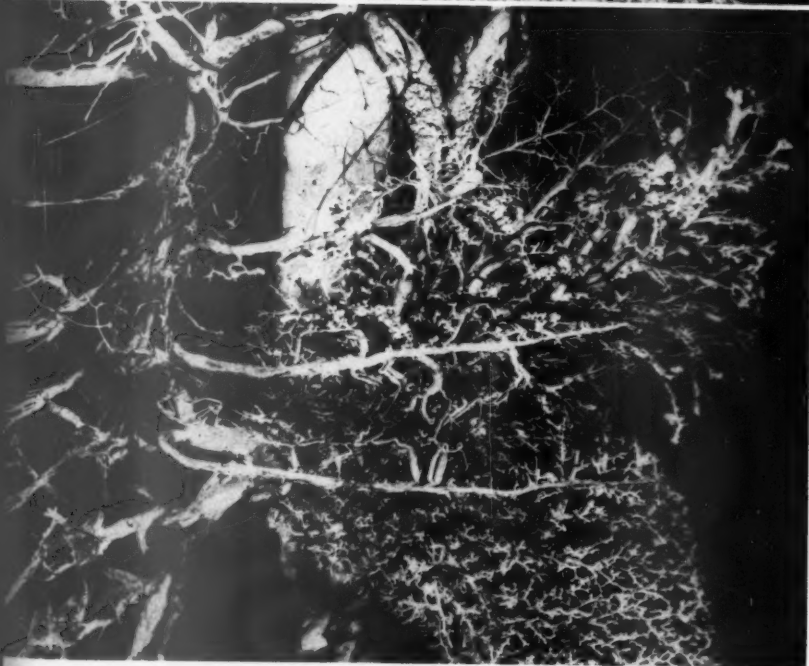
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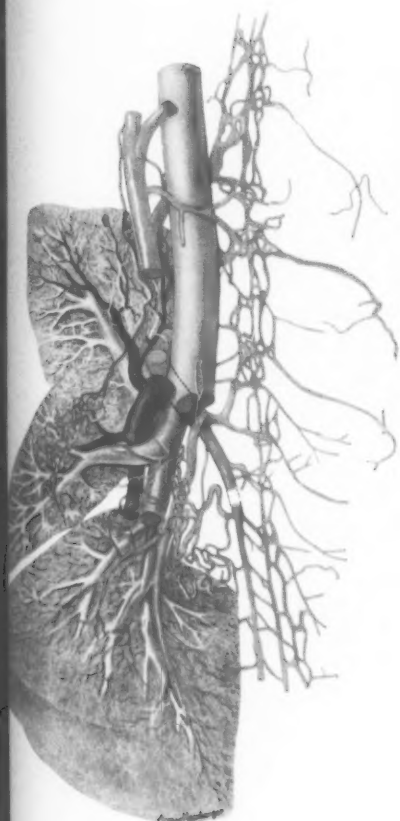
FIG. 7. Drawing to correspond with Figure 4. The connections at many points of the hilar bronchial venous plexus with the pulmonary vein of the lower lobe are shown, as is the ramification of the pericardiophrenic branch into terminal collaterals on the medial aspect of the middle lobe.

FIG. 8. Drawing to correspond with portions of Figures 5 and 6. A segmental pulmonary vein injected from the hilar bronchial venous plexus can be seen more clearly than in Figure 5 in its characteristic fern-like distribution within the superior portion of the right lower lobe.

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FIG. 9. Dog V4. General view of medial aspect of right lung, 5 months after ligation of the pulmonary veins. Hilar bronchial collateral branches derived from: 1. A 5 mm. stem originating from the common trunk of the fourth and fifth right intercostal veins. 2. A trunk, 2 to 3 mm. in diameter, from the posterior aspect of the azygos at the level of the third intercostal space. 3. A descending branch from the left superior intercostal. 4. A pericardiophrenic branch supplying hilar collaterals distributed about the middle lobe. 5. A 4 to 5 mm. branch of the innominate. 6. Small branches from the superior vena cava. 7. Diaphragmatic branches entering the anterior margin of the mediastinal lobe. Key for other labelled structures as in Figure 4.

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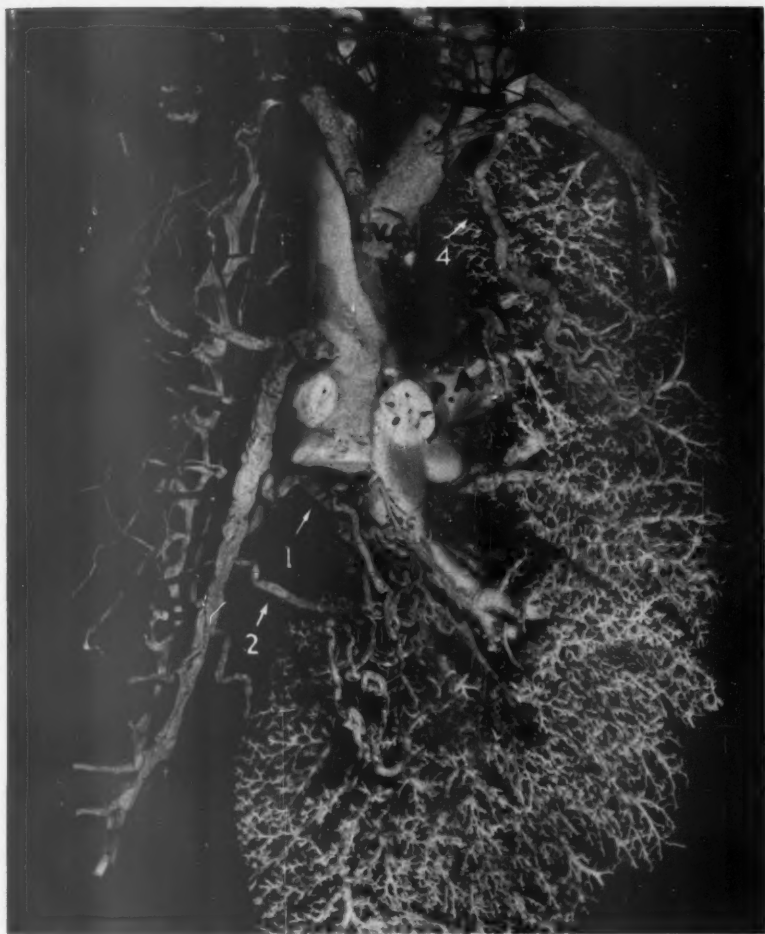


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FIG. 10. Anteromedial aspect of right upper lobe of the cast shown in Figure 9. A photograph and detail drawing serve to demonstrate the end-to-end connections of the large transpleural branch of the innominate (labelled 5 in Figure 9), with branches of the pulmonary veins. The latter are seen to descend into the depths of the lobe toward the hilum.

FIG. 11. Dog V6. Medial aspect of left lung, 4 months after ligation of the pulmonary veins. Hilar collaterals draining left lower lobe derived from: 1. Branch of azygos to fourth and fifth left intercostal veins. 2. Branch from anterolateral aspect of azygos 3 mm. below origin of sixth left intercostal vein. 4. Transpleural branch, 3 to 4 mm. in diameter, of internal mammary that drains the left upper lobe. A small lingular pulmonary vein and the main branch to the first dorsal bronchus of the left lower lobe escaped ligation and were injected with green plastic from the atrium. There has been some injection in green through pre-existing proximal connections with these vessels of portions of the bronchial venous plexus, most of which has been injected in yellow from the azygos.

FIG. 12. Dog V7. Medial aspect of the left lung, 3 months after ligation of the pulmonary veins. A single huge bronchial venous trunk (1) from the azygos branch that is the common source of the fifth and sixth intercostal veins comprises the major hilar collateral drainage for the lower lobe. The drainage of this lobe is supplemented by three smaller transpleural branches, 2, 3, and 4, arising from the anterior aspect of the azygos, below the seventh, eighth, and ninth intercostal veins.

The drainage of the upper lobe is predominantly via terminal collaterals into a huge branch (5) of the brachial vein. Small diaphragmatic branches (7) enter the anterior and medial aspects of the lung.

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FIG. 13. Dog V9. Medial aspect of left lung. Pulmonary veins ligated 5 months previously. Hilar drainage chiefly into a large bronchial vein (1) arising from the left lateral surface of the azygos at the level of the fourth intercostal. The transpleural collaterals are especially well developed in this specimen, *e.g.*, a large branch of the pericardiophrenic (7) and one from the internal mammary vein (8).

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FIG. 14. Dog V10. Anteromedial view of cast of left lung 5 months after ligation of all of the left pulmonary veins. Major hilar collaterals: 3. From the fifth left intercostal vein. 4. From the azygos at a level between the sixth and seventh intercostal veins. Transpleural collaterals: 5. Expanded branches of pericardiophrenic vein. 6. A branch from the superior vena cava.

The major vein of the left upper lobe is connected at the hilum with the expanded hilar bronchial plexus (H), and at the periphery with a transpleural terminal plexus (T).

FIG. 15. General view of lung tissue approximately 4 months after ligation of the pulmonary veins that drained it. The integrity of the parenchyma, absence of fibrosis, hemosiderosis, and thrombosis of vessels are demonstrated. Approximately 100 X.

FIG. 16. Pleura and adhesions. Veins traversing the pleura to enter the pulmonary substance. Their thick muscular walls are appropriate to the size of their lumina.

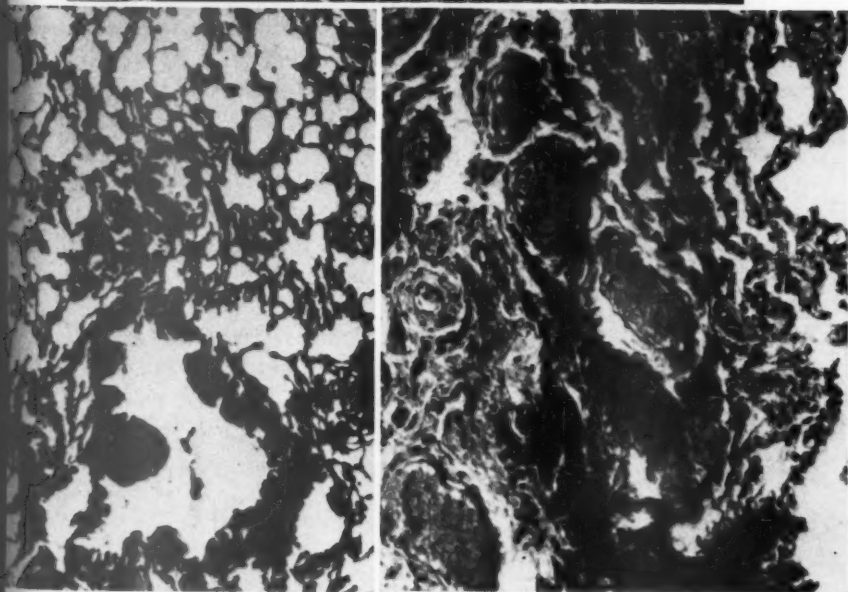
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PNEUMOCONIOSIS FROM EXPOSURE TO KAOLIN DUST: KAOLINOSIS *

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Although the term kaolinosiis occurs in a widely circulated medical dictionary, the name is apparently not used in the few available published reports of pneumoconiosis associated with exposure to the inhalation of dust of clay. At the present time there are only two proved varieties of chronic pulmonary disease developing from the inhalation of particulate matter in the atmospheres of industrial plants or workings: silicosis and asbestosis. The present report sets up a definite disease that may be disabling and may reach an advanced or even fatal stage. While the condition to be described has certain features that are not clearly distinguishable from silicosis, it has other features which warrant the special designation, at least until some particular part of kaolin may be shown to be the disease-causing factor. Inasmuch as there are a few published records of pneumoconiosis occurring in "fuller's earth" workers, such comparisons as seem warranted also will be made with that condition.

In a report by Campbell and Gloyne¹ upon a necropsied case in 1942 in which exposure to the dust of fuller's earth in Surrey, England, was of 38 years' duration, the condition was described as scattered, irregular, black, fibrotic patches from $\frac{1}{4}$ to $\frac{3}{4}$ inch in diameter, not considered to be characteristic of silicotic nodules. Also described is thickening of the walls of the bronchioles, perivascular fibrosis of arterioles, and "reticular" fibrosis of alveoli surrounding dust particles. In the clay concerned in that report montmorillonite was the main constituent while sericite and kaolinite were not present. The soft patchy pneumoconiosis without massive fibrosis differed from silicosis.

McNally and Trostler² found in roentgenologic examinations in 1941 of 49 men working in fuller's earth in Illinois a few with densely mottled areas in the upper lobes of the lungs, but in most of these men there were merely increased bronchial markings.

The few other reports cited by these writers² and the paper by Tonning³ indicate the condition observed as "reticular" fibrosis without silicotic nodulation or massive fibrosis.

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KAOLIN

No attempt will be made here to give a detailed analysis of the composition of kaolin as it occurs in different deposits in various parts of the world, nor to determine whether any particular component of any deposit of kaolin, including free silica (SiO_2), may be the actual offending substance. Perhaps it will be acceptable for the present purpose to define kaolin simply as a kind of clay derived by disintegration of an aluminous mineral such as feldspar or mica. It is commonly called china clay, and in simple terms clay may perhaps be defined as a dispersed system of mineral fragments of hydrated aluminum silicate in which particles smaller than $2\ \mu$ predominate. The term clay refers to a physical condition and not a definite chemical composition. The elements which may be found in kaolin deposits are: silicon (SiO_2), aluminum (Al_2O_3), iron (Fe_2O_3), titanium (TiO_2), calcium (CaO), magnesium (MgO), potassium (K_2O), sodium (Na_2O), manganese (MnO), copper (CuO), and sulfur (SO_3), with water, carbon, and organic matter. The essential constituent of clay or kaolin is the mineral kaolinite, a hydrated aluminum silicate.

The material from which the dust concerned in this study came was apparently derived by open mining and was processed in an industrial plant for distribution and use in various industries. The exposure apparently occurred in such a processing plant. In previous times, before the recognition of such hazards or possible hazards, doubtless some phases of the processing created very dusty atmospheres. So far as our own observations go, those conditions seem to have been largely corrected, but this study shows that wherever workers are exposed sufficiently to inhalation of air heavily laden with particles of kaolin, at least some may develop chronic disease of the lungs as a consequence.

An additional factor that may need to be taken into account is from the addition of chemicals in the course of processing the clay. Those sometimes used are soda ash, trisodium phosphate, and sodium pyrophosphate.

PATHOLOGIC STUDIES

The material concerned in this study consists mainly of the lungs of two men who had worked for considerable periods in a kaolin processing plant. Upon the death of these two persons, necropsies were performed elsewhere and the lungs were sent to us for examination. Subsequently, we were furnished with the chest roentgenograms* of

* The descriptions and interpretations of the roentgenograms are given by courtesy of Dr. H. S. Pettit, Professor of Roentgenology.

each and with a note on the symptoms and duration of occupational exposure.

Case 1

The first case was that of a man, 36 years old, who had apparently worked in the plant for a period of some 17 years. Although it is probable that in the early part of this exposure the atmosphere about his work was heavily laden with dust, at least in the latter part conditions were much improved, and it is believed that his more recent exposure was not particularly hazardous. There was no other suspected dust exposure. No record of the clinical state in this case is available.

The roentgenograms of his chest, taken toward the end of his exposure, were described and interpreted as follows:

Postero-anterior and lateral films of the chest showed a massive confluent consolidation of the upper half of the right lung field, an area of confluent consolidation measuring 8 cm. in diameter in the upper third of the left field, and extensive nodular infiltration of the lower two thirds of the left lung field. The trachea was displaced to the right. No definite area of cavitation was apparent. There was emphysema of the right base. *Summary:* Advanced pneumoconiosis with infection. Extensive confluent consolidation and nodular infiltration.

The description of the portions of the lungs examined by us was as follows:

Gross Observations. The material received consisted of four inadequately fixed portions of lung together weighing 1195 gm. and bearing two large branches of pulmonary vessels containing apparent antemortem thrombi. In one portion of lung there was a roughly wedge-shaped, firm, hemorrhagic area 4 cm. in diameter. The alveolar spaces appeared to be uniformly expanded, producing a vesicular emphysema. There were three areas of whorled fibrous tissue which were of the consistency of heavy tire-tread rubber and presented margins which in some regions were so sharp as to appear encapsulated. These measured up to 7 cm. in diameter and extended from hilum to periphery, where the overlying pleura measured up to 0.5 cm. in thickness. Scattered throughout the remaining pulmonary tissue were many other smaller, but otherwise similar, shot-like fibrous nodules. There were scattered areas with cystic spaces which appeared to be related to inadequate fixation. Hilar nodes were present, heavily pigmented, measuring up to 2 cm. and presenting much the same appearance on the cut surface as the fibrous nodules described, but they were softer.

Microscopic Findings. Nodular areas of whorled collagenous fibrosis contained deposits of brownish black particulate matter. Many alveolar spaces were dilated and their walls were variably attenuated or thickened by a fibrous reaction. In some areas there was fragmentation and clubbing of the alveolar septa and there were pigment-laden macrophages in alveolar spaces as well as within their walls. Some fibrous tissue increase was noted also about blood vessels. One large

vessel contained a recent thrombus and there were hemorrhage and necrosis of an adjacent portion of the lung. The pleura showed a marked fibrous thickening. Lymph nodes showed the same dense fibrous scarring described for the lungs, and heavy pigmentation. When examined under polarized light, innumerable small refractile bodies were seen, yet they were by no means so numerous as the particulate matter visible under ordinary illumination. Lymph nodes presented an entirely similar appearance under polarized light. An additional conspicuous feature was the profound distortion of erythrocytes, practically all of them being of spindle shape (incidental erythrocyte sickling).

The diagnosis recorded from these studies was: *pneumoconiosis (kaolinosis) with pulmonary thrombosis and infarct of the lung.*

Case 2

The second case was that of a man, 35 years old, who had worked in a kaolin processing plant for about 21 years. It is probable that he, too, was exposed to very dusty atmospheres, at least in the early period of his employment. It is reported that 5 years prior to his last illness he had been given a diagnosis of silicosis with suspected concomitant tuberculosis. He had symptoms of dyspnea during his last 3 years, more marked during the final year and extreme for a week or so prior to death. There was associated slight cough productive of small amounts of dark colored sputum, as well as ankle edema at least 3 years before his death. Acid-fast bacilli were reported from the washings of his stomach at that time, but sputum examined shortly before his death was negative for such organisms.

The description and interpretation of his roentgenograms were as follows:

Films of the chest showed a generalized pulmonary emphysema and large confluent areas of consolidation in the upper half of each lung field. The confluent mass in the left hemithorax measured 9 cm. in diameter and its edges were relatively smooth. The two masses in the right upper lung field had a combined diameter of 11 cm. Between them there was an area of cavitation measuring 2.5 cm. in diameter. Below the confluent areas of each lung field there was a fine granular and nodular infiltration. The heart was relatively small. *Summary:* Advanced pneumoconiosis with infection, confluent consolidation, nodular infiltration, cavitation, and emphysema.

The description of the lungs examined by us was as follows:

Gross Observations. A right and left lung, which had been sectioned previously, were received. Within the substance of each lung were hard, nodular, bluish gray areas which might be described as resembling blue marble, and which measured up to 11 cm. in greatest diameter. The right upper and middle lobes were practically replaced by these nodules and the upper half of the right lower lobe was similarly involved. The left lung showed extensive involvement of the upper lobe and of the upper third of the lower lobe. The pulmonary tissue not involved by the large firm nodules showed a pronounced dilatation of the alveolar spaces and a rather diffuse firmness. In the right upper

and middle lobes were cystic spaces with glistening linings, measuring up to 4 cm. in diameter. The pleural surfaces of both lungs were thickened and shaggy, and presented large bullous emphysematous blebs. A major branch of the pulmonary artery in the left lung showed organizing thrombus. Hilar lymph nodes measured up to 2.5 cm., were heavily pigmented, and in part replaced by the same firm, whorled nodularity described in the lungs.

Microscopic Findings. Massive areas of whorled collagenous fibrous tissue were heavily pigmented by deposits of brownish black amorphous material and particulate matter. In some locations alveolar spaces were dilated and some septa showed fibrous thickening while others were attenuated or disrupted. Many spaces were filled with pigment-bearing macrophages and the same sort of material which was widely deposited throughout the fibrous nodules. Cross sections of several branches of the pulmonary artery showed partial or complete occlusion. Pigmented macrophages were scattered through their walls and in the fibrous tissue which occupied the lumina of some. Several of these vessels had crinkled walls suggesting actual compression by the surrounding dense scar tissue. The pleura was markedly thickened and encompassed large bullous spaces. Lymph nodes showed heavy pigmentation and also contained large areas of whorled collagenous fibrous tissue. When examined under polarized light, both the pulmonary tissue and lymph nodes showed a great number of small refractile bodies.

The diagnosis recorded in this case was: *pneumoconiosis (kaolinosi)*.

SUMMARY

While roentgenograms in these two cases apparently do not furnish features upon which definite differentiation between silicosis and kaolinosi may be made, there are two observations of possible significance that may be made from them. The first is that, while the same features may show in cases of silicosis, the massiveness of the involvement of the upper parts of the lungs in kaolinosi is remarkable. Kaolin dust is composed of very fine particles and is a comparatively light dust, possibly having a better opportunity to reach the upper lung areas than heavier dust composed of larger particles. Secondly, although much pulmonary air space is obliterated, emphysema is prominent in the open areas.

The bluish color created by the deposits in the lungs was a distinctive feature. While the reactions causing the development of blue color in clay materials are apparently not clearly understood, it is believed that, because of their adsorptive qualities, clay crystals when mixed

with organic amino compounds may develop a blue reaction under some conditions. Whether this reaction and the consequent development of blue color would occur under all circumstances of deposition of kaolin in the lungs can not be ascertained except from extensive observation, but it should constitute a distinctive feature when it is found, identifying the nature and source of the deposit even if it were not otherwise known.

From the microscopic descriptions it will be noted that while nodular fibrosis with massive whorled collagenous deposits, as in silicosis, composed the more bulky changes, fibrosis of the alveolar walls and thickening and emphysema were conspicuous. This explains the prominence of respiratory difficulties, at least in the one case in which information was available.

Also to be noted especially are the associated vascular lesions. These consisted of extensive obliterative arteritis in which visible particles of foreign material were scattered through the vessel walls, even in the fibrous tissue which occupied the lumina of some arterial branches; and, in the first case, thrombosis of the main branches of the pulmonary artery.

It is regretted that accurate description of the condition of the heart was not available in either case for evidence of the general circulatory effect created by the condition found within the lungs.

CONCLUSION

Sufficient exposure to inhalation of dust of at least some kaolin deposits will cause in some individuals a chronic fibrous disease of the lungs that may be disabling and even prove to be fatal. The term kaolinosis is therefore established as signifying a definite disease state, beyond merely the location of kaolin in the lungs.

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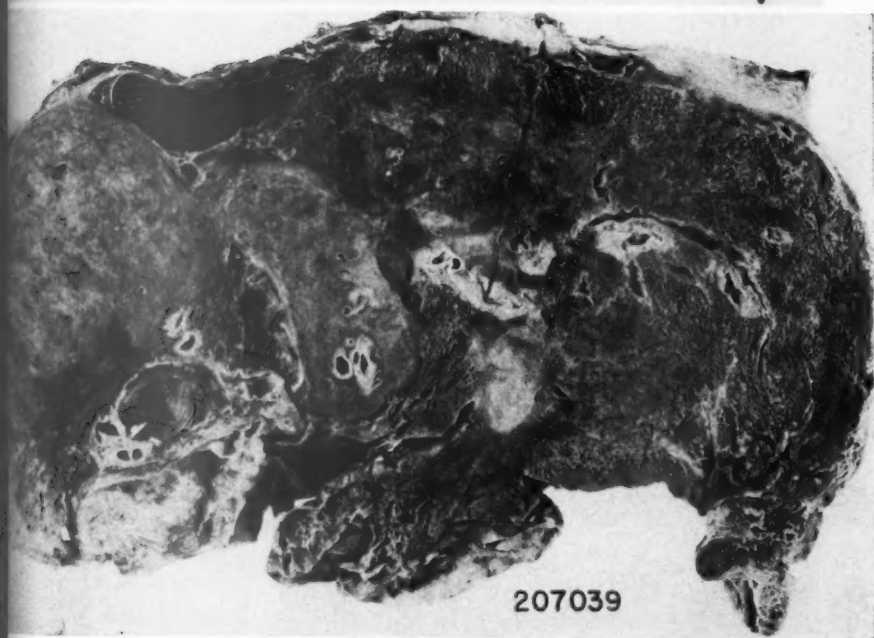
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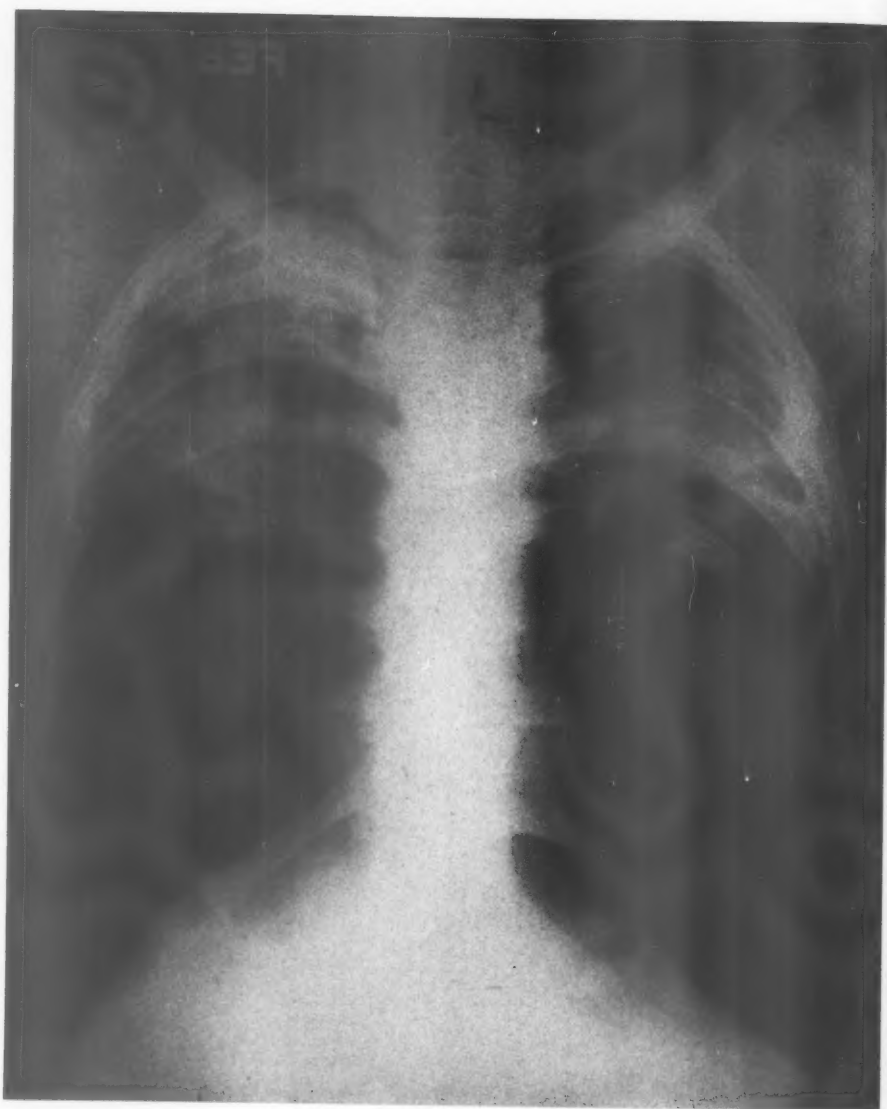
FIG. 1. Sectioned lung, case 1, 156128. Massive nodular pneumoconiosis of upper portion of lung, with diffuse emphysema and pleural thickening.

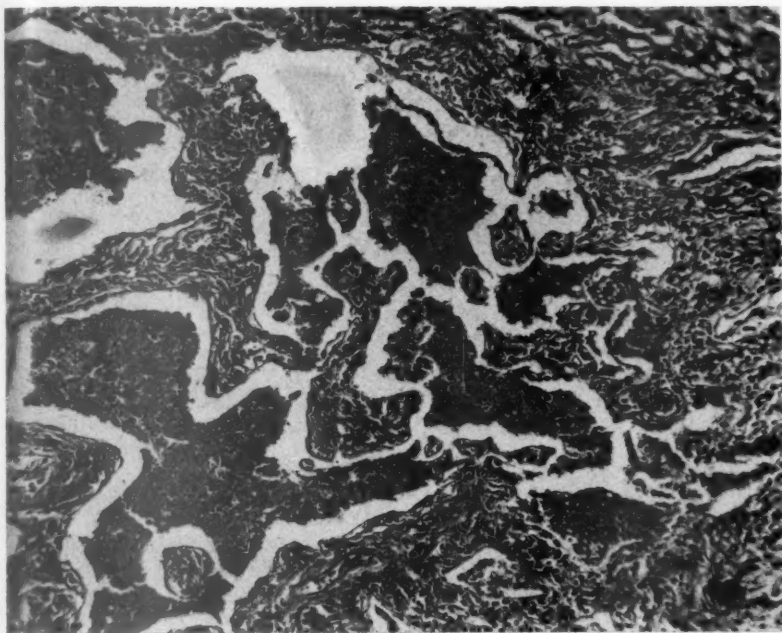
FIG. 2. Sectioned lung, case 2, 207039. Massive nodular pneumoconiosis of upper portion of lung, large subpleural cavity, diffuse emphysema, and pleural thickening.

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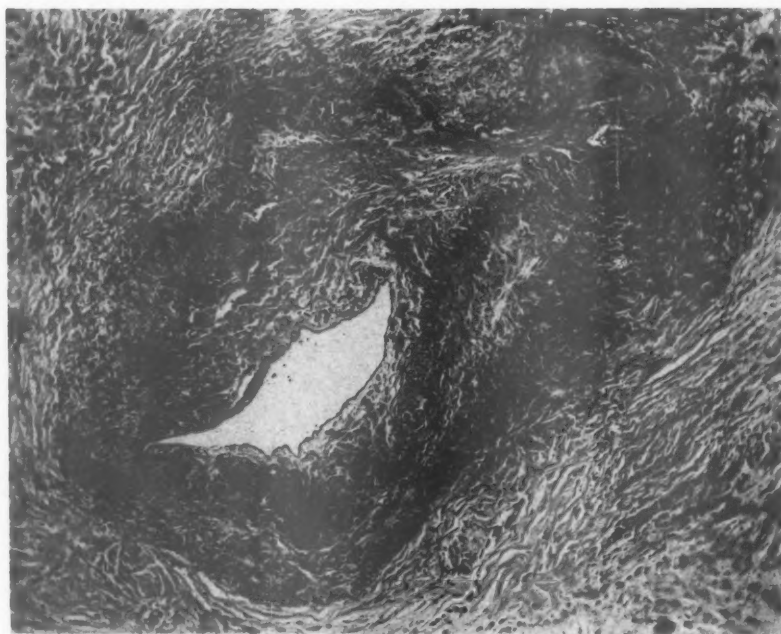
FIG. 3. Chest roentgenogram, case 2. Massive upper confluent pneumoconiosis.

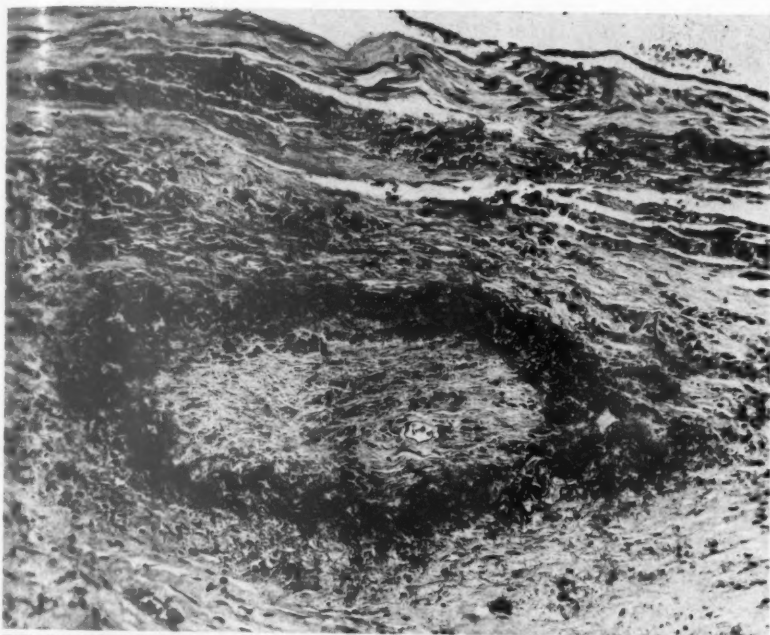
FIG. 4. Case 1. Massive accumulation of dust-laden macrophages and debris in alveoli, with surrounding fibrosis. $\times 125$.

FIG. 5. Severe vascular lesion, with perivascular and intimal fibrosis. $\times 100$.

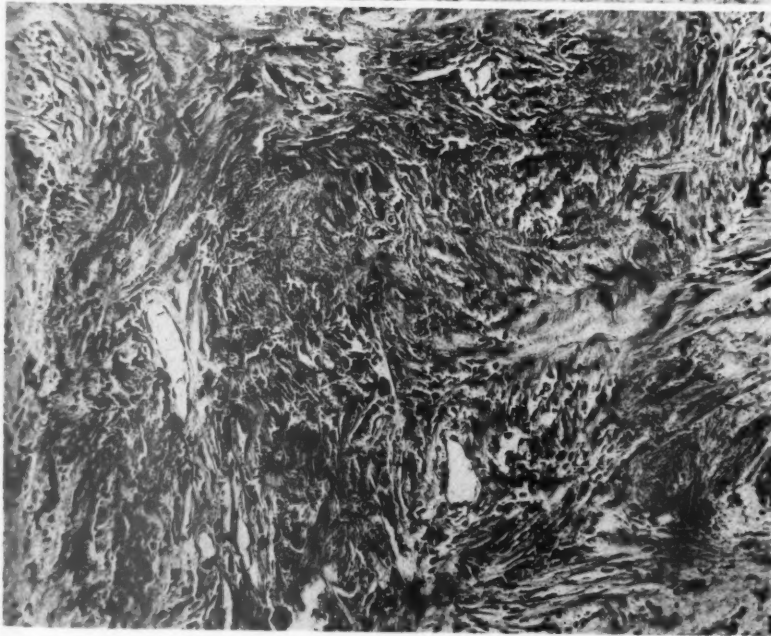
FIG. 6. Perivascular and obliterating intravascular fibrosis. $\times 125$.

FIG. 7. Dense hyalinizing fibrosis with alveolar obliteration and dust deposit. $\times 125$.





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ARTERITIS IN GUINEA-PIGS, PRODUCED BY EMBOLI OF COTTON, RESEMBLING THE ARTERITIS OF HYPERSENSITIVITY *

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In a previous report¹ the effects of cotton emboli in the pulmonary arteries of the human and rats have been described. Later, Konwaler² and Jaques and Mariscal^{2a} reported the finding of cotton emboli in several human cases and Wartman, Hudson, and Jennings³ published their results of the injection of filter paper particles into the pulmonary circulation of rabbits. Filter paper is composed of cotton with some linen added. Wartman and associates described a foreign body reaction located in the intima, media, or adventitia and believed that the fibers could pass through the wall or lodge within the wall of the artery.

Based on the observation that cotton fibers did not produce arteritis when injected into the pulmonary arteries of the rat, it occurred to us that this material might be utilized in a study of the antigen-antibody reaction. By the injection of antigen-impregnated cotton into the venous circulation, antigen could be placed directly against the wall of the pulmonary artery of an animal previously sensitized to the same antigen; thus it would be possible to localize and study in greater detail any resulting vascular lesion. Accordingly the following experiment was undertaken.

PROCEDURE AND METHODS

Absorbent cotton was prepared for injection as previously described.¹

Guinea-pigs were sensitized by the subcutaneous injection of 1.0 cc. of physiologic saline solution that contained 1 mg. of purified crystalline egg albumin per cc. Four injections were given with an interval of 3 to 5 days between injections. In the beginning, skin tests were done to determine the sensitivity of each animal so injected. The skin tests were later abandoned as it was noted that the animals were always rendered hypersensitive and also because no correlation was found between the degree of hypersensitivity and the intensity of the arteritis that was produced after the cotton was injected.

Purified egg albumin was coupled to the disodium salt of 2 naphthol-3:6 disulfonic acid (R salt), according to the method described by Heidelberger, Kendall, and Soo Hoo.⁴ The coupled R salt-egg albumin contained 13.2 mg. of egg albumin per cc. This preparation was used

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as the cotton seemed to be dyed by the solution and the color would give a rough index of the amount carried by the cotton. The physiologic saline solution in which the finely chopped cotton had been sterilized was withdrawn with a sterile syringe and then replaced with approximately 2 cc. of the R salt-egg albumin solution. The cotton was allowed to soak in this solution, in a refrigerator, for periods varying from 4 to 48 hours. The cotton assumed a deep purplish red color. Before injection into the animals the excess R salt-egg albumin solution was removed without disturbing the mass of cotton at the bottom of the tube and the R salt-egg albumin impregnated cotton was resuspended in 5 to 7 cc. of sterile physiologic saline solution. With this method, when 1 cc. of the saline solution containing suspended cotton was injected, there remained enough of the R salt-egg albumin attached to the cotton and free in the solution to cause mild shock in a few instances.

Other sensitized animals were injected with sterilized cotton that had been soaked in physiologic saline solution containing 13.2 mg. of purified crystalline egg albumin per cc. The egg albumin solution was removed and sterile physiologic saline solution (5 to 7 cc.) was added to the cotton before injections were made.

Under light ether anesthesia the jugular vein was exposed. One cc. of the saline solution containing the suspended cotton was injected into the vein. The wound was closed with interrupted sutures of silk.

The animals were sacrificed at varying intervals 12 to 48 hours after being injected. Specimens of the various organs were fixed in Zenker's solution without the addition of acetic acid or formalin and in 10 per cent formalin solution. Ten serial sections were cut from each block of lung embedded in paraffin; every other section of each series was stained with Weigert's stain for elastic tissue and counterstained with hematoxylin and eosin. In some instances sections of lung were stained with Mallory's phosphotungstic acid hematoxylin. Sections were made from other organs also.

RESULTS

Except for mild shock in some of the animals, the injections gave rise to no symptoms. The lesions produced were identical in the animals that had been injected with cotton soaked in R salt-albumin solution and in those that had received cotton soaked in the solution of egg albumin.

The gross changes in the lungs were in direct relation to the amount of cotton injected. Tiny bright red hemorrhages were seen beneath the

pleura and at times on the cut surface; the greater the amount of cotton, the more numerous were the hemorrhages.

After a lapse of 12 hours, lesions were found in the walls of the pulmonary arteries and arterioles. Adjacent to the cotton emboli, small segments of the media were necrotic. The necrotic muscle cells were swollen, deeply stained with eosin, somewhat rigid in appearance, and the nuclei had disappeared. The necrosis of the muscle was sometimes in the inner part of the media just outside the elastica interna, at other times in the outer portion of the media. In those arterioles in which necrosis was more extensive, it did not involve the media of the entire circumference. Foreign body granulomas were beginning to form about some of the cotton particles. Collected about these vessels, the walls of which were damaged, were large mononuclear cells, pseudo-eosinophils, and variable numbers of extravasated red blood cells.

At the end of 24 hours the lesions were more pronounced; the entire media was often necrotic near the cotton and medial necrosis was seen in arterioles that contained cotton about which no foreign body granuloma had formed. Where granulomas had formed, the wall of the vessel was often stretched. The large mononuclear cells collected about the vessels were frequently spread apart by edema fluid, and beyond them were often hemorrhage and pseudo-eosinophils in variable numbers. Large mononuclear cells were beginning to penetrate between the necrotic muscle cells. An occasional multinucleated giant cell was found among the other cells.

When the animal was allowed to live 48 hours the arterial lesions were well developed. In many of the arteries and arterioles necrosis of the media was complete near the cotton, and about the vessel was the cellular reaction. The necrotic muscle was at times fused into a homogeneous mass; in other instances the necrotic muscle cells remained separate. The removal of the necrotic muscle of the media had progressed. The invading large mononuclear cells spread the necrotic medial cells apart and pushed them outward. In many of these vessels only a few fragments of the necrotic media could be found, in others no trace of the media could be discovered. The large mononuclear cells followed the direction of the medial cells and so they came to lie in a circular or concentric fashion, filling the space between the internal and external elastic lamellae. The lesions in a single animal were usually not all of the same stage and it was frequently possible in a single section of lung to follow the progress of the arteritis from the early stage to that of replacement of the necrotic media by the large mononuclear cells (Figs. 1, 2, and 3).

In all of these damaged vessels it was clearly seen that the elastica interna was still intact, though it might be bent or pushed outward by a fragment of cotton indenting the wall of the vessel; it was not beaded or swollen. Fragments of the elastica externa were found but this layer is not normally so distinct as is the internal elastica. The endothelium had not undergone necrosis though it had often proliferated when a fragment of cotton lay against it.

Some of the granulomas contained fibrin but it was not possible to demonstrate fibrin in the necrotic media nor in the surrounding zone of inflammatory reaction. Bacteria could not be demonstrated in sections stained by Gram's method.

These lesions were found only at the sites where the cotton had lodged. The necrosis of the media extended a short distance proximally and distally to the cotton. At the various time intervals, some fragments of cotton were seen in vessels without any lesion in the adjacent media and with or without a granuloma. When the cotton had come to rest in the precapillary portion of the arteriole, the reaction usually was that of a foreign body granuloma. Large mononuclear cells collected about the vessel with an occasional pseudo-eosinophil, but this inflammatory reaction was much less intense than that about the involved arteriole. In one animal that received a very large injection of cotton, early infarction of a small area of the lung had resulted.

The arteritis was not found in any organ other than the lung.

In this series of 65 animals, arteritis was present in 38 (58.5 per cent).

CONTROLS

The control series consisted of 44 guinea-pigs; 4 of these had been made sensitive to egg albumin and were injected with untreated sterile absorbent cotton. Forty animals were not sensitized: 4 received untreated cotton; 25, cotton that had been soaked in the R salt-egg albumin solution; and 11, cotton that had been soaked in a solution of egg albumin. In many instances the same preparation of cotton was used for the injection of sensitized and normal animals. The control animals were allowed to live for 12 to 48 hours after injection.

Arteritis was found in all groups of the control animals at the sites where the cotton had lodged in the pulmonary arterial system. This arteritis was, in every respect, similar to the arteritis in the sensitized animals that received cotton soaked either in the solution of R salt-egg albumin or in the solution of egg albumin (Figs. 4, 5, and 6). The arteritis was present in 25 of the 44 control animals (56.8 per cent).

DISCUSSION

There are obvious differences in the effect of emboli of cotton in the pulmonary arteries and its branches as seen in rats and in guinea-pigs. In the rat the reaction was that of formation of a foreign body granuloma, without any demonstrable necrosis of the vessel wall. The granuloma was extruded through a rent in the wall of the arteriole and finally came to lie outside the vessel. The wall of the arteriole was restored by a new formation of connective tissue, a new elastica interna and, after several weeks, new muscle was being formed. In the guinea-pigs, the outstanding change was necrosis of the media of the arterioles and arteries that began 12 hours after the injection of cotton fibers. The necrosis appeared first in localized areas of the media, sometimes situated in the inner part, in other instances in the outer portion. Soon the necrosis involved the entire circumference of the media and extended a short distance beyond the cotton. About the vessel a zone of edema frequently spread apart the accumulated large mononuclear wandering cells. Peripheral to these were extravasated red blood cells and pseudo-eosinophils, at times in large numbers. The experiments were not continued beyond 48 hours; therefore, no data are at hand as to the later changes in the vessel wall.

It is apparent that there is no relationship between previous sensitization and the arteritis, as an identical lesion was found in the control animals. Equally obvious was the lack of an association between the foreign body granuloma and the damage to the wall of the arteriole and artery. Numerous instances were observed of a fragment of cotton with associated necrosis of the media without any granuloma having been formed about the cotton. Except for some stretching of the vessel, no other detectable effect of the granuloma could be determined.

The arteritis produced in the guinea-pigs by cotton closely resembles the description of the changes in the vessels in hypersensitive animals recorded by Gerlach⁵ and in the experiments of Vaubel,⁶ Masugi and Sato,⁷ Rich and Gregory,⁸ McKeown,⁹ Ehrich, Seifter, and Forman,¹⁰ and Hopps and Wissler.¹¹ In these experiments protein substances were used as antigens.

Arteritis has been recorded in experiments and case reports as the result of sensitization to chemicals, as, for example, iodine and sulfa derivatives (Rich,¹² Lichtenstein and Fox,¹³ French,¹⁴ and More, McMillan, and Duff¹⁵).

Arteritis has been described also in experiments in which there appears to be a relationship between hypertension and the arteritis

(Friedman, Jarman, and Klemperer,¹⁶ Selye and Pentz,¹⁷ Smith, Zeek, and McGuire¹⁸). Byrom and Dodson¹⁹ and Waters and de Suto-Nagy²⁰ concluded from their experiments that the hypertension need not be sustained but only to exist over short periods to bring about the arterial lesions.

Conceivably, the injection of cotton could have raised the blood pressure in the pulmonary arterial system when the animal received a considerable amount of cotton that was widely distributed throughout the pulmonary arterial tree. However, in several of the animals the amount of cotton injected was very scant and it was necessary to study many sections of the lungs to find an occasional fiber. It would be difficult to maintain that so few fibers could have resulted in an increase of blood pressure in the pulmonary arteries and have caused the lesion. Furthermore, if these lesions were the result of an increase of blood pressure, it would be reasonable to expect that the arteritis would be found in vessels where cotton had not lodged; the arteritis, however, was found only in the immediate vicinity of a piece of cotton.

Byrom and Dodson¹⁹ concluded that sudden overstretching of the vessel was important in the development of the arteritis associated with hypertension, the overstretched wall undergoing necrosis. The effect of localized overstretching of the vessel where the cotton lodged is doubtful. Granulomas at times had formed about the cotton fibers; they distended the lumen and stretched the vessel wall without any necrosis of the media. In contrast, a piece of cotton, without a granuloma, often was observed in the lumen of an arteriole; the vessel lumen was not widened, the wall was not stretched, and yet the media was necrotic. It is not likely that a possible increase of blood pressure or overstretching of the vessel wall could be responsible for the arteritis found in these guinea-pigs.

An important question with relation to the arteritis is whether cotton or some of its products can be an antigen. Prausnitz²¹ has stated that in byssinosis there was hypersensitivity to a substance that could be extracted from cotton dust. He believed that allergy was a factor in the disease. He also believed that the dust contained an unidentified irritant or toxic fraction that may be different from the fraction considered to be the cause of the hypersensitivity. Cayton, Furness, and Maitland²² have concluded from their investigations that specific generalized hypersensitivity is unlikely to be the cause of byssinosis and that the late reaction produced by the inoculation of the dried extract of cotton dust was almost certainly a direct toxic effect on the tissues.

There is evidence that oxidized cotton may be an antigen. It has been shown that cotton may be oxidized by nitrogen dioxide to prod-

ucts containing varying percentages of carboxyl. These would therefore contain cellobiuronic acid units, separated by glucose, at intervals in the cellulose chain. Heidelberger and Hobby²³ noted a close relationship between the polysaccharide of oxidized cotton and those of type III and type VIII pneumococci. They have demonstrated that extracts of oxidized cotton, at high dilutions, precipitated antipneumococcus type III and type VIII horse sera and that the oxidized cotton with 16 per cent carboxyl corresponds more closely in its immunologic behavior to type VIII substance than to type III polysaccharide.

The possibility was considered that oxidation of the cotton may take place during the preparation of absorbent cotton with the formation of the specific polysaccharide. The method of preparing absorbent cotton was carefully studied; oxidation of the cotton may take place during bleaching but this would be trifling. Should this arteritis be due to some antigenic material in the cotton, the reaction to it must be unusually rapid. There would have to be sensitization of the vessel wall, formation of antibody, reaction of antigen and antibody, with early necrosis of the media within 12 hours. Evidence is lacking that this reaction could take place in so short a period of time.

As noted, there are some who believe there is a toxic fraction in extracts of cotton that has a direct effect on the tissues. To eliminate a possible toxic fraction some absorbent cotton was placed in a Soxhlet apparatus and extracted with water for 4 hours, then with isopropyl alcohol for 7 hours to remove any fatty or waxy materials remaining in the cotton. The cotton was then prepared and sterilized as outlined and when injected intravenously into 4 guinea-pigs the same arterial lesion was produced in the pulmonary arteries in 2 of the animals.

If the arteritis was due to a toxic fraction of the cotton, it might be anticipated that the initial damage would be to the endothelium with the formation of a thrombus, and later the media might become necrotic. This, however, is not the sequence of events. The endothelium at the site of the arteritis does not appear to have undergone necrosis, and thrombi do not form. The absence of thrombosis about the cotton is one of the striking features of the lesions in the human, the rat, and the guinea-pig. The initial change in the arteries of the guinea-pig is necrosis of the media beginning in some instances in the inner part, at other times in the outer portion of this layer. A possible diffusible toxic substance in cotton, that may have a more damaging effect on smooth muscle than on endothelium, cannot be eliminated, though from these experiments no evidence to support this can be adduced.

The removal of the necrotic muscle of the media by the large mono-

nuclear cell was unusually rapid and began after 24 hours. At the end of 48 hours, in numerous instances, the necrotic muscle had been completely removed and the space, formerly occupied by it, was filled by large mononuclear cells, these latter cells being arranged as the muscle had been. McKeown⁹ described the invasion of the necrotic media by mononuclear cells in hypersensitive rabbits and this is illustrated (Fig. 16) in the report of Ehrich, Seifter, and Forman.¹⁰ In our studies the experiments have not been continued for a sufficient time to indicate what may take place later in the restitution of the damaged media of the vessels.

CONCLUSIONS

When injected into the venous circulation of guinea-pigs, cotton fibers produce lesions in pulmonary arteries, arterioles, and precapillary branches at the sites where the fibers lodge.

Foreign body granulomas form about many of the particles of cotton.

Necrosis of the media is found in the arterioles and arteries. This necrosis begins at the end of 12 hours. About the damaged arteries and arterioles large mononuclear cells and pseudo-eosinophils collect, and often red blood cells are extravasated. The necrotic muscle is rapidly removed by large mononuclear cells and the removal is frequently complete at the end of 48 hours. The medial necrosis also occurs in vessels that contain cotton about which no foreign body granuloma has formed. It has not yet been determined what factor or substance is responsible for the arteritis. The lesion in the pulmonary vessels, induced by the cotton, closely resembles the arteritis that has been described in hypersensitive animals.

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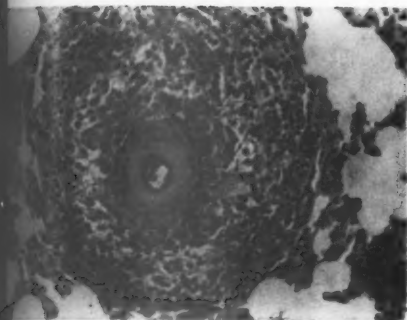
[Illustrations follow]

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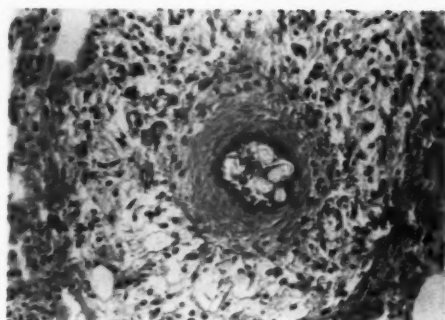
Each animal was sacrificed at the end of 48 hours. The sections were stained with Weigert's elastic tissue stain and with hematoxylin and eosin.

- FIG. 1. Guinea-pig no. S4. Sensitized by the subcutaneous injection of purified egg albumin. Cotton soaked in R salt-egg albumin solution was injected into the jugular vein. The media of the pulmonary arteriole is necrotic at the site where the cotton has lodged. Large mononuclear cells have collected about the vessel and removal of the necrotic media has begun. There is a zone of edema about the vessel and beyond this some hemorrhage and pseudo-eosinophils. The elastica interna is intact. $\times 131$. (For comparison with Fig. 4.)
- FIG. 2. Guinea-pig no. S4. The lumen of the arteriole is distended by particles of cotton. The elastica interna is intact. The media is necrotic. About the vessel are large mononuclear cells and removal of the necrotic media has begun. The zone of edema is wide and in this area is some hemorrhage; at the periphery of this zone are a few pseudo-eosinophils and hemorrhage. $\times 131$. (For comparison with Fig. 5.)
- FIG. 3. Guinea-pig no. S4. About the cotton in the lumen of the pulmonary arteriole is a foreign body granuloma that distends the lumen. The elastica interna is stretched but unruptured. The necrotic media has been replaced by large mononuclear cells arranged concentrically between the internal and external elastic lamellae. There is only slight edema about the vessel and at the periphery of this area are many pseudo-eosinophils. $\times 147$. (For comparison with Fig. 6.)
- FIG. 4. Guinea-pig no. 142. Non-sensitized control. Cotton soaked in R salt-egg albumin solution injected into the jugular vein. Except for a multinucleated giant cell near the vessel, the changes in the pulmonary arteriole are identical with those described in Figure 1. $\times 131$.
- FIG. 5. Guinea-pig no. 155. Non-sensitized control. Untreated cotton was injected into the jugular vein. The changes in the pulmonary arteriole are the same as those described in Figure 2 except the edema about the vessel is not so marked. $\times 131$.
- FIG. 6. Guinea-pig no. 155. Non-sensitized control. The lesion in the pulmonary arteriole is identical with that shown in Figure 3. $\times 147$.

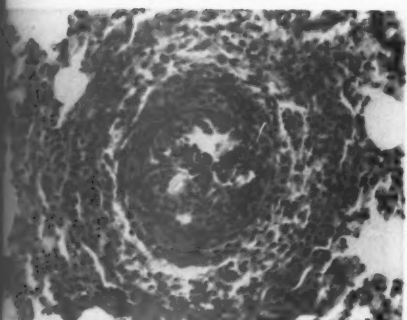
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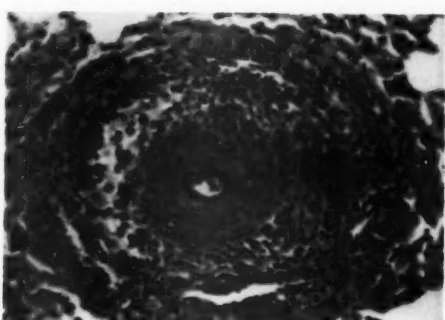
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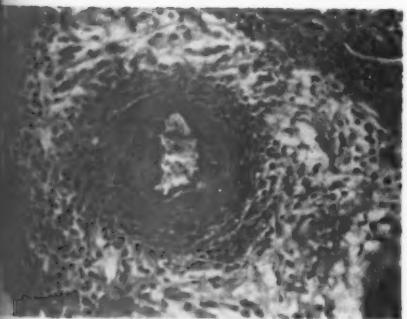
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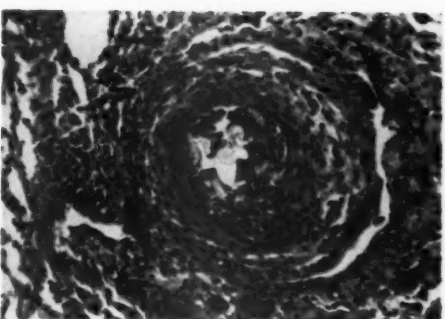
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RENAL DISEASE IN HYPERPARATHYROIDISM *

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The interrelation of renal disease, hyperparathyroidism, and generalized osteitis fibrosa is still imperfectly understood. The present paper records three examples of different types of hyperparathyroidism, each associated with organic and functional renal changes: (1) secondary to chronic pyelonephritis and producing an extreme degree of osteitis fibrosa; (2) secondary to chronic pyelonephritis and producing, besides osteitis fibrosa, severe metastatic calcification; and (3) a primary parathyroid adenoma with secondary bony and renal changes.

REPORT OF CASES

CASE I. HYPERPARATHYROIDISM SECONDARY TO CHRONIC RENAL DISEASE PRODUCING SEVERE OSTEITIS FIBROSA

H. F. was a male, 25 years of age. Four years earlier, while on active duty in the Army, he developed pain in the right thigh. Examination revealed a fusiform swelling of the left radius and a similar swelling in the upper third of the right femur. Roentgenologic examination of these bones showed expansion and rarefaction. Intravenous pyelography revealed very small kidneys which failed to concentrate the dye. The blood pressure was 120/80 mm. of Hg and the fundi were normal. The urine contained small amounts of protein (38 mg. per 100 ml.) and some red cells. The biochemical findings, summarized in Table I, led to a diagnosis of hyperparathyroidism secondary to chronic renal insufficiency.

For 3 years he remained reasonably fit and active. He then began to complain of backache, and roentgenograms showed a collapsed third lumbar vertebra. The blood urea had risen to 340 mg. per 100 ml., but clinically he was not uremic. He remained ambulant for another year, though limping slightly with his right leg.

Six months later (*i.e.*, 4½ years after his first symptom) he entered the hospital for the last time. He was ill, anemic, and barely able to walk. For 2 weeks he had suffered from anorexia, vomiting, bad taste in the mouth, bleeding from the gums, and increasing drowsiness. The skeletal lesions had increased and he had a third swelling at the upper end of the right humerus. The peripheral arteries showed calcification radiographically. The blood urea was 580 mg. per 100 ml. At no time was there a record of abnormal blood pressure. The fundi were still normal. The testes were atrophic. His general condition deteriorated, and he died in uremic coma 2 weeks after admission.

Necropsy Findings

The body was that of a slightly built, poorly nourished young man, 160 cm. in length. The chest was rachitic, with beading of the lower costochondral junctions. The cranial bones were 1.9 cm. in thickness, and of the type sometimes referred to as "Pagetoid." The surface

* Received for publication, March 2, 1954.

below the periosteum was rough and porous, and the pattern of cortex and diploë was destroyed, and replaced by a thick, uniform layer of osteoid tissue that a saw cut through as it would dry rot. The left radius (Fig. 1) and right femur were similarly affected, the whole bone being slightly pliable and compressible between the fingers. In addition, the right femur showed a well marked coxa vara and a fracture just below a large "cyst" (*i.e.*, cavity filled with soft tissue) 3.3 cm. in

TABLE I
Biochemical Investigations in Case 1*

	Normal values	10-7-46	8-11-48	10-18-49	11-28-49
Total serum calcium [Ca], mg./100 ml.	9-11	9.9	7.8	9.4	9.9
Plasma phosphates [PO ₄], mg./100 ml.	3-4.5	5.3	8.8	7.2	8.5
Total serum proteins, gm./100 ml.	6-8	8.1	7.2	6.3	6.3
Alkali reserve, m.eq./l.	25-35			18	14
Blood urea, mg./100 ml.	20-40	177	340	495	580
Serum alkaline phosphatase in King-Armstrong units					
Urine protein, mg./100 ml.	3-13	26	49	41	44
Urea clearance, % of normal	0	38	40	60	
	100%	11%	5%	4%	
Ionic serum calcium [Ca ⁺⁺] [†]	4.25-5.25	4.0	3.2	4.5	4.7
[Ca] × [PO ₄]	30-45	55	69	68	84
[Ca ⁺⁺] × [PO ₄]	14-21	21	28	32	40

* Patient died December 3, 1949.

† Method of McLean and Hastings (1935).

diameter. Roentgenograms of these specimens showed extreme rarefaction and loss of the normal lines of stress.

Only two parathyroid bodies were found. The right upper showed hypertrophy (12 by 6 by 2 mm., 102 mg.). It retained the color, shape, and general pattern of a normal parathyroid gland, though greatly increased in size. The right lower gland (10 by 10 by 20 mm., 1110 mg.) was firm, white, and compact and macroscopically resembled an adenoma (Fig. 2).

The kidneys were both contracted and finely granular (combined weight, 85 gm.). The capsules were slightly adherent and the cortex greatly narrowed (Fig. 3). Radiopaque material lay along the line of the corticomedullary junction (Fig. 4). When cut across, several hard crystalline masses were found embedded in the parenchyma or surrounded by a soft brownish substance. On analysis, the calculi unexpectedly proved to consist of calcium sulfate, while the brown substance was largely organically combined iodine, presumably re-

sulting from intravenous pyelography 2 years earlier. (Iodine content of kidney substance, 115 mg. per 100 gm.)

The heart weighed 11 oz. and showed slight left ventricular hypertrophy. The aorta, on the other hand, was hypoplastic. Some of the peripheral arteries showed medial calcification. The tonsils were ulcerated and contained calcareous deposits. The thyroid gland was pale and atrophic, the pituitary body enlarged (800 mg.), and the adrenal glands normal. Both testes were notably atrophic. The alimentary tract, liver, and pancreas showed nothing of interest.

Histologic Examination

Kidneys. The kidneys showed chronic pyelonephritis with retention of calcium sulfate (Fig. 5). Nearly all recognizable glomeruli showed varying degrees of fibrosis, from thickening of the basement membrane of Bowman's capsule to complete hyalinization. Both large and small arteries were conspicuously thickened, but the afferent arterioles were involved only very occasionally.

Superimposed was a deposit of transparent crystals (calcium sulfate) in considerable quantities. These crystals resisted ordinary stains for calcium salts, but were anisotropic. Groups of small crystals were seen in the distal convoluted tubules, in some cases mixed with albuminous debris. Similar deposits were seen in larger spaces, partly or wholly denuded of lining epithelium (Fig. 6). The largest crystalline masses were in the form of small concretions embedded in dense fibrous tissue.

These crystals were distributed largely along the boundary zone between cortex and medulla, and were not found in the glomeruli, diseased or healthy, nor in the collecting tubules of the medulla. Calcium deposits of the amorphous basophilic type were not observed. The anisotropic material presumably represented a glomerular filtrate which had crystallized out in the tubules as a result of resorption of fluid. Incapable of further passage in the urine, the crystals had led to blockage and distention of certain renal tubules. Degeneration and desquamation of the lining epithelium had occurred, and finally small concretions had formed, embedded in fibrous tissue.

Parathyroid Glands. The hypertrophy of the right upper parathyroid gland was due wholly to chief cell proliferation, the cells being slightly larger than normal, in respect to both nuclei and the cytoplasm. Water-clear cells were not observed, and oxyphilic cells were few and widely separated. The gland had the same septate lobulation as a normal gland, but it differed in the complete absence of fat. Close inspection

of the apparently solid sheets of cells revealed an underlying alveolar pattern, in places frankly acinar; the lumina contained red blood cells, although the surrounding tissue was free of interstitial hemorrhage (Fig. 8). Several more compact foci were seen throughout, compressing the surrounding gland (Fig. 7). These appeared to be the "germinative centers" of Erdheim.

The "adenoma" of the right lower gland, despite macroscopic differences, was remarkably similar microscopically to the right upper parathyroid gland.

Bone. In the left radius and right femur, the picture was that of advanced osteitis fibrosa (Figs. 9 and 10). Where hemopoietic foci had survived, they showed a normoblastic reaction, with prominent megakaryocytes. The "cyst" in the right femur contained fat, hemopoietic tissue, and hemorrhage, but osteoclastomatous tumors were not observed.

In the costochondral junctions bone and cartilage were splayed out along an irregularly interdigitating line of union, with osteitis fibrosa on the one side and irregular nodules of cartilage on the other. The appearances differed from true rickets, the cartilage cells being completely disorderly and arranged in clumps as in a chondroma (*cf.* Brockman, 1927).

The testes showed advanced atrophy of the seminiferous tubules. The interstitial tissue appeared to be increased, but this might have been a false impression due to a general relaxation accompanying shrinkage of the tubules. The epididymides, near the vasa, were normal, but the ductuli efferentes in the first part were filled with anisotropic crystals, similar to those in the kidneys, but insufficient in quantity to allow chemical analysis.

The increase in weight in the pituitary body was clearly attributable to hypertrophy of the pars anterior, where the eosinophilic cells were unusually prominent and showed an increase in cytoplasm rather than in numbers. The thyroid vesicles showed epithelial atrophy and increased colloid.

The liver, spleen, pancreas, and adrenal glands were normal.

CASE 2. HYPERPARATHYROIDISM SECONDARY TO CHRONIC RENAL DISEASE PRODUCING SEVERE METASTATIC CALCIFICATION

L. H., a female splint-maker, died at the age of 22 after an illness of 2 years. Previous illnesses were scarlet fever at the age of 10, not complicated by clinical nephritis, and recurrent sore throats when she was 13. Since the age of 20 she had suffered from alternating periods of irregular bleeding and of amenorrhea. Six months after her first symptom, she developed nocturnal thirst and frequency. Three months later she noticed a lump over the back of the second right metacarpal, which persisted for a few months and then disappeared; others followed

and remained. These began as tiny nodules below the skin, later becoming soft and cystic but not painful or tender, and interfering little with movement.

The second year of her illness began with lassitude, dyspnea on climbing stairs, and frontal headaches, and she was admitted to Westminster Hospital for examination. At that time she appeared ill, with pale mucous membranes, dry sallow skin, and furred tongue. The right middle and left index fingers showed slight clubbing. A remarkable feature was the presence of multiple subcutaneous swellings on the backs of the hands, around both wrists and elbows, over the front of each leg, on the thorax, and one, measuring 5 by 7.5 cm., over the vertebra prominens. Small shotty nodules, 1 to 2 mm. in size, were palpable in the cheeks and the forearms.

The swellings on the backs of the hands were cystic and unattached to skin or tendons (Figs. 11 and 12). When the fist was clenched, small yellow plaques stood out from the rest of the skin. One dusky blue swelling collapsed after aspiration of serosanguineous fluid and did not recur; others remained stationary for weeks until a further phase occurred when they enlarged and became temporarily painful. The joints were unaffected till the late stages of the disease when the size of the swellings interfered with fine movements of the hands.

The blood pressure on admission was 210/130 mm. of Hg, but the heart was not enlarged, and the fundi were normal. Chvostek's and Trousseau's signs were not elicited. The urine was pale, of fixed specific gravity of 1008 to 1010, and contained 350 mg. of protein per 100 ml. Chemical study of the blood (Table II) showed gross nitrogen retention with high plasma phosphates and serum alkaline phosphatase, but with normal calcium level. The two calcium \times phosphate products calculated were both well above normal limits.

Following blood transfusion, the patient improved sufficiently to be discharged, but dyspnea and nocturnal frequency increased, the swellings in the hands enlarged, and new ones appeared over the lower ribs. Intermittent claudication of both legs occurred. On re-admission 1 month before her death, the blood pressure had fallen to 130/90, and the biochemical changes were accentuated. She died in uremia shortly afterwards. The changes in chemical findings in the blood during the 6 months preceding death are recorded in Table II. The principal one was a gradual

TABLE II
*Biochemical Investigations in Case 2**

	Normal values	7-6-42	8-7-42	9-21-42	1-21-43
Total serum calcium [Ca], mg./100 ml.	9-11	9.1	10.5		9.5
Plasma phosphates [PO ₄], mg./100 ml.	3-4.5	6.0	7.9		8.3
Total serum proteins, gm./100 ml.	6-8	6.0	6.6		6.3
Alkali reserve, m.eq./l.	25-35			19	15
Blood urea, mg./100 ml.	20-40	242	198		281
Serum alkaline phosphatase in King-Armstrong units	3-13	58		44	32
Urine protein, mg./100 ml.	0	350	+		+
Urea clearance, % of normal	100%		6%		3%
Ionic serum calcium [Ca ⁺⁺] [†]	4.25-5.25	4.3	4.8		4.5
[Ca] \times [PO ₄]	30-45	55	83		79
[Ca ⁺⁺] \times [PO ₄]	14-21	26	38		37

* Patient died February 2, 1943.

[†] Method of McLean and Hastings (1935).

rise in the plasma phosphate level with increasing acidosis. The calcium \times phosphate products remained high throughout. The calcium and phosphorus balance was investigated in July, 1942, but no significant departure from the normal was observed.

Necropsy Findings

The body was that of a poorly nourished, undersized adult female with pale, puffy features. Irregular swellings about the hands and fingers and in the thoracic wall contained a creamy calcareous substance. The skeleton was not deformed. The skull cap was uniformly thickened up to 1.5 cm. and showed the "Pagetoid" appearance referred to in case 1. The inner ends of the clavicles, the vertebral bodies, and the pelvic bones showed similar softening, while costochondral junctions 1, 7, 8, and 9 on the left and 1 on the right were enlarged.

The kidneys were regular in shape and reduced in size to 85 gm. each. The capsules stripped easily, revealing a finely granular surface. On section the cortex was reduced but the corticomedullary junction was clearly defined.

All four parathyroid bodies were enlarged (left upper, 16 by 8 by 5 mm.; left lower, 20 by 15 by 8 mm.; right upper, 4 mm.; and right lower, 18 by 10 by 5 mm.). All were buff colored, but the left upper also contained darker areas. Their total weight was not recorded, but the combined weight of the left upper and lower bodies was 2.44 gm.

The heart weighed 420 gm., due to left ventricular hypertrophy. A hard, calcareous mass was found in the ventricle in relation to the posterior mitral cusp. Both the coronary and systemic arteries showed patchy thickening and calcification.

Other organs: The brain contained multiple small hemorrhages in the pons. The lungs, gastro-intestinal tract, liver, spleen, pancreas, pituitary, and adrenal glands showed nothing of note.

Histologic Examination

Kidneys. The kidneys showed diffuse fibrosis. Most of the glomeruli were in varying stages of fibrosis, as in case 1. The medullary tubules contained cellular and granular casts, while the mucosa of the calyces showed chronic ulcers. Thickening of the arteries was observed, with calcification of the internal elastic lamina in the larger ones, but the afferent arterioles were not affected.

The striking feature was the presence of quantities of amorphous calcium salts, staining dark blue with hematoxylin. Throughout the cortex, calcium was deposited in the thickened capillary loops of effete glomeruli and in the fibrous ring replacing Bowman's capsule (Fig.

14). It was not detected in the more healthy glomeruli, but was abundant in the distal convoluted tubules. It was not detected in the lumina, or in the cytoplasm of the degenerate lining epithelium, or along the line of the tubular basement membrane. Calcium was not observed in the collecting tubules. Non-staining anisotropic crystals were present in very small amount in the distal convoluted tubules, mixed with albuminous material or in the interstitial tissue. They bore little anatomical relation to the amorphous calcium salts, and their nature is unknown. These changes were interpreted as chronic pyelonephritis with metastatic calcification of the tubules. The calcium in the fibrotic glomeruli may be the result of the fibrosis and not the cause, *i.e.*, increased serum calcium, incapable of filtration by the sclerotic tufts, had been deposited in their walls.

Parathyroid Glands. Only the left upper parathyroid gland, and one of those on the right, were examined. The former showed, throughout half of its substance, chief cell hyperplasia incorporating a few small foci of oxyphilic cells and a nodule 1.5 mm. in diameter composed wholly of oxyphilic cells. The other half of the gland was an adenomatous nodule 6 mm. in diameter, with columnar-celled acini, some of which contained red blood cells (Fig. 15). The right gland had the same features as the solid part of the left one.

Bones. The changes in the bones were those of generalized osteitis fibrosa, as in case 1.

Terminal Phalanges. Under the nail-beds the bone was expanded into a network of small cysts separated by fibrous septa. The proximal half of the phalanx showed osteoporosis only.

CASE 3. PRIMARY HYPERPARATHYROIDISM DUE TO AN ADENOMA PRODUCING OSTEOPOROSIS AND RENAL INSUFFICIENCY

The patient, an elderly married woman, attended Westminster Hospital intermittently over a period of 7 years up to the time of her death at the age of 70. When first seen she complained of breathlessness on effort, had edema of the ankles, and a blood pressure of 180/80 mm. of Hg. An electrocardiogram showed left axis deviation. Over the next 2 years she lost weight, and the skin became dry and pigmented. The blood pressure was then 210/110 and a catheter specimen of urine contained a small quantity of albumin and occasional red blood cells. The blood urea was 39 mg./100 ml., the serum cholesterol 213 mg./100 ml., and there was no evidence of renal insufficiency.

She continued to lose weight, and 6 years after the onset of symptoms she weighed only 83 lbs. She still had albuminuria and edema of the ankles and the blood pressure was 180/90. She developed a slow fibrillation and was admitted to the hospital with early cardiac failure. Blood urea at that time was 64 mg./100 ml. and an intravenous pyelogram revealed distention of the renal calyces. Roentgenograms of the skeleton suggested the diagnosis of hyperparathyroidism. Chemical

examination of the blood (Table III) showed high serum calcium and alkaline phosphatase with low plasma phosphates and some impairment of renal function. The calcium \times phosphate products were normal. These findings appeared to favor a primary adenoma with secondary renal involvement, and in view of the latter, Dr. Donald Hunter advised against exploring the neck, the prognosis in such cases

TABLE III
*Biochemical Investigations in Case 3**

	Normal values	10-28-40	11-10-40	12-9-40	7-28-50	10-17-52
Total serum calcium [Ca], mg./100 ml.	9-11		13.7	14.4	14.3	19.2
Plasma phosphates [PO ₄], mg./100 ml.	3-4.5		2.9	2.8	2.6	5.0
Total serum proteins, gm./100 ml.	6-8		5.7	6.5	6.8	5.7
Alkali reserve, m.eq./l.	25-35					
Blood urea, mg./100 ml.	20-40	64	61		39	157
Serum alkaline phosphatase in King-Armstrong units	3-13		34	20	60	33
Urine protein, mg./100 ml.	0		25	+	+	+
Urea clearance, % of normal	100%		24%			
Ionic serum calcium Ca ⁺⁺ †	4.25-5.25		7.2		6.6	12.0
[Ca] \times [PO ₄]	30-45		40		37	96
[Ca ⁺⁺] \times [PO ₄]	14-21		21		17	60

* Patient died October 20, 1950.

† Method of McLean and Hastings (1935).

being poor. He further considered that the hypertension, though possibly due to renal involvement, was more likely to be coincidental.

The patient improved sufficiently to be discharged, but returned 9 months later complaining of lethargy, coldness, and vomiting. The blood urea had risen to 157 mg. and the serum calcium to 19.2 mg. per 100 ml., so that the calcium \times phosphate product was now raised. The symptoms increased in severity and the patient died in coma 3 days later.

Numerous catheter specimens of urine over the last 5 years were always acid, contained albumin, occasionally red blood cells and pus cells, but never casts or crystals. On three occasions during the last year, lactose-fermenting coliform bacilli were isolated from catheter specimens. Earlier cultures were sterile.

Necropsy Findings

The body was that of an emaciated and elderly female, showing a brownish pigmentation of the skin. The hair of the scalp was falling out and the axillae and pubis were hairless. The skeleton showed generalized osteoporosis, the bones being soft and spongy. Three parathyroid bodies were found, the right lower and left upper being of normal appearance and dimensions. The right upper had the appearance of an adenoma and weighed 7250 mg. The cut surface was pale gray and contained a central cyst. There was no thyroid tissue.

The kidneys both showed granular contraction. The capsules were adherent to the surface, and on section gritty particles were encountered in the corticomedullary zone. The liver showed a fine cirrhosis. The heart was dilated in all four chambers, the left ventricle being hypertrophied. The arteries showed general atherosclerosis.

Death was due to bronchopneumonia.

Histologic Examination

Parathyroid Adenoma. In the plane of sectioning, two thirds of the tumor consisted wholly of chief cells, and it contained a large cyst, 2 by 0.5 cm. The other one third was almost wholly oxyphilic. Both areas were subdivided by thick fibrous septa, imparting a lobulated appearance. The chief cells were slightly larger than normal, and some had abundant non-staining cytoplasm, although smaller than the large water-clear cells of primary hyperplasia. Closer inspection of the chief cells revealed an alveolar pattern, which in places became frankly acinar with cubical cells arranged around lumina or small cysts containing an albuminous colloid substance. The large central cyst appeared to have been formed by confluence of smaller cysts.

The oxyphilic portion was more homogeneous and without cysts. Small groups of chief cells were scattered throughout and the numerous transitional forms on the margins of these foci indicated a fundamental identity between the two cell types. At one edge of the section, compressed by an expanding nodule, was a thin rim of what appeared to be normal parathyroid gland (Fig. 16).

Kidneys. The renal picture was that of chronic pyelonephritis. In the outer part of the cortex were fibrotic wedges of tissue showing glomerular atrophy and gross distention of the subcapsular spaces (Fig. 13), while the intervening convoluted tubules were reduced to small canals filled with albuminous fluid (Fig. 17). Large parts of the inner cortex were unaffected and the typical pattern of granular contraction was not observed. A few of the small arteries were thickened. Basophilic amorphous calcium salts were present in moderate quantities along the corticomedullary junction, affecting the distal convoluted tubules and the loops of Henle. Deposits were seen in the lumen, mixed with epithelial debris, or, more frequently, between the tubular epithelium and its basement membrane. In places, the epithelium had sloughed, leaving naked calcareous deposits projecting into the lumen. The glomeruli did not contain calcium salts. A very few anisotropic crystals were seen, either in the lumina of the distal convoluted tubules or in their lining epithelium. This was interpreted as chronic pyelone-

phritis of fairly recent origin, presumably due to damage or blockage of convoluted tubules by metastatic calcification.

Left Radius. The cancellous portion of the left radius showed osteoporosis, the bony trabeculae being thin and widely separated by normal fatty marrow.

The liver showed fine cirrhosis with evidence of cell damage toward the periphery of the lobules. There was no intracellular pigment.

The pars anterior of the pituitary gland showed hypertrophy of the eosinophilic cells similar to that of case 1. The spleen, pancreas, and suprarenal glands were normal.

DISCUSSION

Symptomless increase in the size of all four parathyroid bodies in association with various forms of chronic renal disease has long been recognized (Bergstrand, 1921; Pappenheimer and Wilens, 1935; Gil-mour, 1947). The increased weight in such cases is not more than 50 to 100 per cent of the normal parathyroid weight. According to Albright and Reifenstein (1948), renal insufficiency leads to phosphate retention with a rise in blood phosphate and a consequent fall in blood calcium. It seems likely from animal experiments that the parathyroid glands undergo *compensatory hyperplasia* in response to the low blood calcium level (Drake *et al.*, 1937; Ham *et al.*, 1940; Duguid, 1942).

Clinical hyperparathyroidism, however, is rare in chronic renal disease. When it does occur, it is usually in children, adolescents, or young adults with a long history of renal insufficiency, and takes the form of osteitis fibrosa, with or without metastatic calcification. The renal insufficiency in such cases has been variously ascribed to congenital lesions—renal hypoplasia or malformations of the urinary tract—or to some type of nephritis. The total parathyroid tissue may be 100 times the normal weight. The course of events would appear to be an exaggeration of the effects described in the preceding paragraph, but in addition Snapper (1949) stressed the rôle of prolonged acidosis.

Good accounts of the bony manifestations—renal osteitis fibrosa or renal osteodystrophy, as the condition is now called—were given by Brockman (1927), Langmead and Orr (1933), Shelling and Remsen (1935), and Howard (1938). Most of these were in adolescents, but similar (if milder) lesions have been recorded in children, and even in infants in the first few months of life (Andersen and Schlesinger, 1942; Schellack, 1939).

In another group of cases, again mostly adolescents, the bony changes were accompanied, or even overshadowed, by the deposition of large calcareous masses in the neighborhood of joints, as in the re-

ports of Hubbard and Wentworth (1920-21), Smyth and Goldman (1934), and Magnus and Scott (1936). Albright, Drake, and Sulzowitch (1937) recorded a case in a man 45 years old, and described the lesion as "the adult counterpart of so-called renal rickets." In a review of published cases, Herbert *et al.* (1941) stressed the importance of a raised calcium \times phosphate product in the blood in relation to metastatic calcification, and suggested the following sequence of events. Chronic renal disease leads to retention of phosphate, hyperphosphatemia, and hypocalcemia, as usual. If this state of affairs lasts long enough, parathyroid hyperplasia occurs, and the resultant hyperparathyroidism leads to mobilization of calcium from the bones, either initiating osteitis fibrosa, or aggravating already existent bone disease. The serum calcium is raised from its previously low level to normal or above normal; this, in conjunction with the pre-existing hyperphosphatemia, raises the calcium \times phosphate product, thus over-saturating the blood and tissues and leading to metastatic calcification.

A disconcertingly similar osteitis fibrosa, metastatic calcification, and renal fibrosis may also be found in *primary hyperparathyroidism*, due either to a tumor of one of the parathyroid bodies, or to primary hypertrophy of all of them. The excess of parathyroid hormone leads to hyperphosphaturia, hypophosphatemia, hypercalcemia, and hypercalcuria, in that order (Albright and Reifstein). A direct effect of the hormone on bone may also be concerned (Dent, 1953). Later, secondary renal damage may occur, causing phosphate retention and hyperphosphatemia. Both the calcium and the phosphate levels in the blood are now high, and metastatic calcification occurs, notably in the kidneys. There are three ways in which the kidneys may be implicated in primary hyperparathyroidism: (1) renal calculi, with or without chronic pyelonephritis; (2) nephrocalcinosis, in which the calcium is deposited in the substance of the kidneys (discussed later); (3) Albright and Reifstein believed that hypercalcuria and hyperphosphaturia alone can damage the renal tubules, causing polyuria and polydipsia and simulating diabetes insipidus.

Differential Diagnosis in Parathyroid Disorders

It is thus apparent that there may be considerable difficulty in distinguishing between chronic renal disease, causing secondary hyperparathyroidism, and primary hyperparathyroidism, causing secondary renal disease. The distinction is an important one, since primary adenomas usually call for surgical removal. Various differential diagnostic criteria have been formulated, clinical, biochemical, and pathologic.

Clinical. Prolonged renal insufficiency in adolescents accompanied

by the stigmas of "renal rickets" (stunted growth, beading of costochondral junctions) suggests renal origin. Massive deposits of calcium below the skin appear to occur only in secondary hyperparathyroidism. A history of acute nephritis in infancy is usually lacking; and, more often than not, hypertension, cardiac hypertrophy, and changes in the fundi are absent. In primary hyperparathyroidism, on the other hand, renal function is initially normal, but insufficiency may develop later (see case 3).

Biochemical. A very high serum calcium value (above 15 mg./100 ml.) or a high serum calcium with low serum phosphate, suggests primary hyperparathyroidism. In the secondary type the levels are reversed, the phosphate being high and the calcium low in the early stages, though the calcium may rise later to normal or above, while the hyperphosphatemia is sustained (as in case 2). The urinary excretion of calcium is typically increased in primary hyperparathyroidism and normal or diminished in the secondary type. This is a particularly useful diagnostic feature, as it usually persists even in advanced cases in which the usual chemical features of the blood may have become obscured by secondary changes, as has been noted.

Pathologic. Biopsy of one of the enlarged parathyroid bodies may yield valuable information, although it is questionable whether the differential diagnosis is as clear-cut as some accounts suggest. There are three broad types of enlargement (Albright and Reifstein; Woolner, *et al.*, 1952): (1) primary tumor (adenoma, single or multiple; carcinoma); (2) primary hypertrophy; (3) secondary hyperplasia in chronic renal diseases.

Primary hypertrophy involves all four glands, and the microscopic picture of the uniformly large "water-clear" cells is so characteristic that it cannot be confused with any other type of parathyroid enlargement. Its etiology is unknown (Albright, Drake, and Sulkowitch; Castleman and Mallory, 1935; Woolner, *et al.*).

On the other hand, despite statements to the contrary, secondary hyperplasia, especially when one of the glands is conspicuously larger than the others (Gilmour) is not always readily distinguishable microscopically from a primary adenoma. This is illustrated in our cases 2 and 3, in which the differences are rather in degree than quality. Thus, a secondarily enlarged gland might, from the biopsy specimen alone, be mistaken for an adenoma, and there are two cases in the literature (Johnson, 1939; Downs and Scott, 1941) in which this appears to have happened.

Renal Lesions in Primary Hyperparathyroidism

Records are few and data scanty in respect to renal lesions in primary hyperparathyroidism. Reference has been made to Albright and Reifenstein's view that hyperphosphaturia alone can damage the renal tubules, in the absence of nephrocalcinosis, but this is based on clinical and chemical data plus negative roentgenologic appearances. The precise nature of the renal damage is not known.

Renal lesions hitherto demonstrated at necropsy have been associated with calcium deposits, either in the renal parenchyma (nephrocalcinosis) or in the form of calculi with or without chronic pyelonephritis, not specific in type and therefore not considered here.

The interrelation of nephrocalcinosis and structural damage is not clearly understood. Albright, Aub, and Bauer (1934) stated that precipitates of calcium phosphate in the renal tubules lead to secondary contraction and insufficiency, but they did not indicate how the lesion evolves. Anderson (1939) believed that calcification is preceded by parenchymal damage, and that deposition of calcium salts is secondary to tubular necrosis. Calcareous deposits then accumulate in the interstices of the medulla, sometimes bulging into the lumina of the collecting tubules and causing further damage in the form of obstruction, tubular distention, and cyst formation.

Experimentally, nephrocalcinosis can be induced in animals by injecting parathyroid hormone (Hueper, 1927; Learner, 1929). In later experiments this has been followed by a type of granular contraction broadly comparable to that seen in human hyperparathyroidism (Chown, Lee, and Teal, 1936; 1937; Duguid, 1933-34; 1936; 1938). In human patients dying from uremia preceded by severe vomiting of gastro-intestinal origin, acute tubular degeneration and calcification are not uncommon. In a series of unpublished experiments on pyloric obstruction in rats causing calcium nephrosis, carried out by one of us (A. D. M.) in 1939, it was concluded that the calcium deposits and the tubular degeneration were not congruent changes, and although due to a common cause, were probably independent phenomena.

Less is known about the late effects of calcinosis in humans, but in the light of the foregoing, our case 3 is instructive. The slow development of renal insufficiency was followed over a period of 5 years. At necropsy the kidneys showed calcinosis and an unusual type of patchy fibrosis. Hypertension was not a prominent factor and it is reasonable, therefore, to assume that the wedges of "chronic pyelonephritis" (to

use a much abused term for want of a better) were the result of prolonged nephrocalcinosis.

Renal Lesions Causing Secondary Hyperparathyroidism

It is fairly obvious that, in the past, many cases were diagnosed as renal rickets which would today be called renal osteodystrophy (Snapper) or renal osteitis fibrosa (Albright, Drake, and Sulkowitch). Moreover, some authors have used the terms renal rickets and renal dwarfism interchangeably; though Evans and Evans (1950), while admitting their common etiology, reserved the term renal dwarfism for children in whom infantilism is predominant. It is thus clear that there is considerable overlapping, clinical and pathologic, between renal dwarfism, renal rickets, and renal osteodystrophy. There is justification, therefore, for considering the published literature on these three syndromes together.

Barber (1926), in reporting 8 necropsies on cases of renal dwarfism, described the renal lesion as "pure interstitial nephritis." Ellis and Evans (1933) recorded 14 necropsies in renal dwarfs, and in almost every case, irrespective of age and sex, they found small fibrous and often granular kidneys, dilatation of renal pelves and ureters, and hypertrophy of the bladder. No obstruction to urinary outflow could be demonstrated, and the authors concluded that the lesions were due to a neuromuscular defect, probably congenital. The microscopic features of the kidneys were not recorded.

Derow and Brodney (1939) briefly reviewed the literature on the renal lesions in renal rickets. These fall into three categories: (1) small fibrotic kidneys described as chronic glomerulonephritis, chronic interstitial nephritis, or chronic pyelonephritis; (2) congenital malformations of the kidney (polycystic disease, renal hypoplasia); (3) obstructive lesions of the urinary tract (hydronephrosis, renal calculi, congenital dilatation of the ureters, collar-neck obstruction of the urinary bladder, congenital valves or deformities of the urethra, and even phimosis).

Most authors who employ the term chronic nephritis do so with diffidence, stressing that the lesion is not identical with that of Bright's disease (Greene, 1922; Hamperl and Wallis, 1933; Price and Davie, 1936-37). Mitchell (1930) observed granular and fibrous kidneys in infants shortly after birth, and suggested intra-uterine inflammation as the cause. Coplin (1917) believed that congenital hypoplasia might render the kidneys liable to some form of nephritis.

In recent literature, under the heading secondary hyperparathyroidism, the patients have generally been older, and the kidneys have

usually been described as small and fibrous, with a few small cysts and often with calcareous particles embedded in the parenchyma along the corticomedullary junction, with pelves, ureters, and bladder of normal dimensions (see cases 1 and 2). Hydronephrosis or hydro-ureter have been recorded (Smyth and Goldman, Hubbard and Wentworth, Shelling and Remsen). Polycystic disease has been found in adult cases (Gutman, Swenson, and Parsons, 1934; Nelson, 1937). The microscopic appearances have been described as chronic glomerulonephritis (Langmead and Orr; Magnus and Scott; Albright, Drake, and Sulko-witch; Snapper); chronic pyelonephritis (Shelling and Remsen); and congenital hypoplasia of the kidneys (Herbert *et al.*). Congenital hypoplasia, however, is to some extent a hypothetical diagnosis, since the early stages of the lesion are seldom seen, and the original appearances are likely to be modified by superimposed calcinosis, just as fibrosis follows the calcinosis of primary hyperparathyroidism; indeed the differences between the fibrous calcinosis of primary and secondary hyperparathyroidism are not sufficiently striking to be of diagnostic value at necropsy.

There are, however, other arguments in support of a congenital lesion. Besides polycystic disease and malformations of the urinary tract, congenital anomalies of other organs may be found at necropsy (Greene); and on several occasions the disease has afflicted two members of the same family (Barber, 1913; Bader, 1934; Chown, 1935-36).

It may be noted that the father of the patient described as our case 1 died of "kidney disease," while the patient's elder brother succumbed to chronic renal insufficiency at the age of 27. We are obliged to Sir Horace Evans and Dr. J. F. Smith of the London Hospital for details regarding the brother. Over a period of years he had recurrent renal colic, albuminuria, a gradually rising blood urea, and mild hypertension. At necropsy the kidneys were granular and contracted, and the calyces contained brown pultaceous material. A section shows changes similar to our case 1, though milder, and lacking the deposits of calcium sulfate. The bones and parathyroid glands were normal.

To avoid possible confusion it should be mentioned here that a true "renal rickets" or osteomalacia may result from a renal tubular lesion as a result of impaired reabsorption of phosphate and/or bicarbonate. This condition is usually called renal acidosis or nephrocalcinosis (Fanconi, 1936; Lightwood, 1935; Latner and Burnard, 1950) and appears to be quite distinct from the conditions already considered. The literature on this syndrome has been reviewed by Albright and Reifens-stein (1948), and more recently by Milne *et al.* (1952) and Anderson *et al.* (1952).

Metastatic Calcification

Both primary and secondary hyperparathyroidism can produce metastatic calcification upon occasion and the renal effects of this process have been considered. It seems reasonably certain that this tendency is associated with a rise in the solubility product for calcium phosphate in the serum, and much work has been done on the most suitable way of calculating this product from the comparatively crude serum estimations (Herbert *et al.*; Albright and Reifstein). We have used only two of the simplest procedures: (a) the crude product of plasma inorganic phosphorus and total serum calcium both expressed as mg. per 100 ml. (normal limits about 30 to 45 in adults, 40 to 60 in children); (b) the product of plasma inorganic phosphorus and ionized serum calcium, the latter being calculated from the total serum calcium and protein concentration by the nomograph of McLean and Hastings, 1935 (normal limits about 14 to 21 in adults, 19 to 28 in children). In our cases the second product gave a slightly better correlation with the clinical findings, as it was initially normal in cases 1 and 3 with little calcification and was uniformly high in case 2 with much calcification.

It is, of course, generally recognized that a high calcium \times phosphate product is not invariably associated with metastatic calcification and it is likely that such conditions must exist for a considerable period before calcification ensues. In addition, the state of acid-base balance may well be of significance. While calcification can occur both with acidosis and with alkalosis, the well known effect of acidosis in increasing the ionization of calcium salts and also the solubility of calcium phosphate need consideration. From existing information it is impossible to predict whether acidosis *per se* would favor calcification or not.

SUMMARY

Three cases of hyperparathyroidism associated with renal fibrosis in adults are described. The criteria for distinguishing secondary hyperparathyroidism from primary hyperparathyroidism causing renal disease are examined in the light of our own experience and of published cases. The following conclusions are reached:

The macroscopic and microscopic appearances of a primary parathyroid adenoma do not differ very sharply from some forms of secondary hyperplasia.

Likewise the gross and microscopic findings in the kidneys do not differ greatly in the two diseases. Renal fibrosis in primary hyperparathyroidism is probably secondary to metastatic calcification.

Renal fibrosis in secondary hyperparathyroidism generally is not due to glomerulonephritis. It is sometimes due to congenital causes, but the picture is confused by secondary calcinosis.

Metastatic calcification is associated with a high calcium \times phosphate product in the serum but may also be influenced by disturbances of acid-base balance.

Our thanks are due to Dr. W. E. Lloyd, Dr. S. P. Meadows, and Mr. H. E. Lockhart-Mummery for clinical data; for some of the necropsy findings we are obliged to Drs. R. M. Haines and G. D. Lumb. We are especially indebted to Dr. R. W. Parnell for the clinical notes on case 2, and permission to use photographs. Dr. A. F. Hallimond, late of the Geological Survey and Museum, kindly reported on the crystals in case 1. We also thank Dr. Peter Hansell of the Medical Photography Department at Westminster Hospital, and Mr. J. F. Wilson for histologic preparations.

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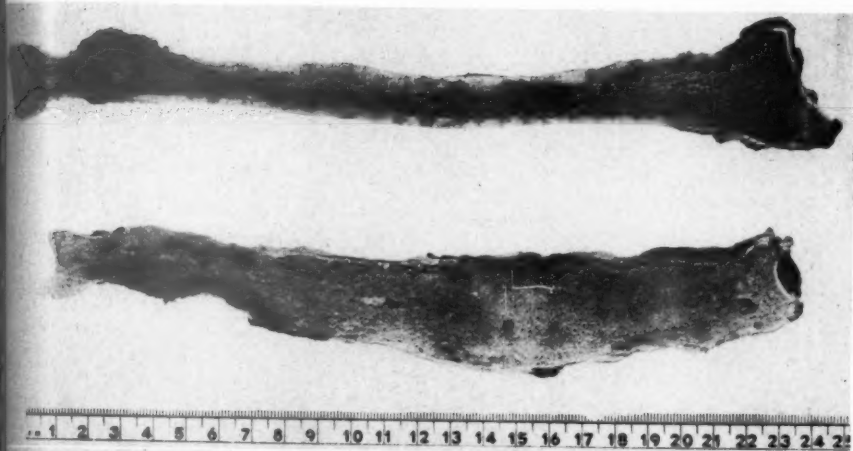
[Illustrations follow]

LEGENDS FOR FIGURES

- FIG. 1. Case 1. Left radius cut longitudinally to show advanced osteitis fibrosa.
Normal control above.
- FIG. 2. Case 1. Thyroid gland and "adenoma" of right lower parathyroid gland.

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FIG. 3. Case 1. Kidneys showing granularity and fibrosis.

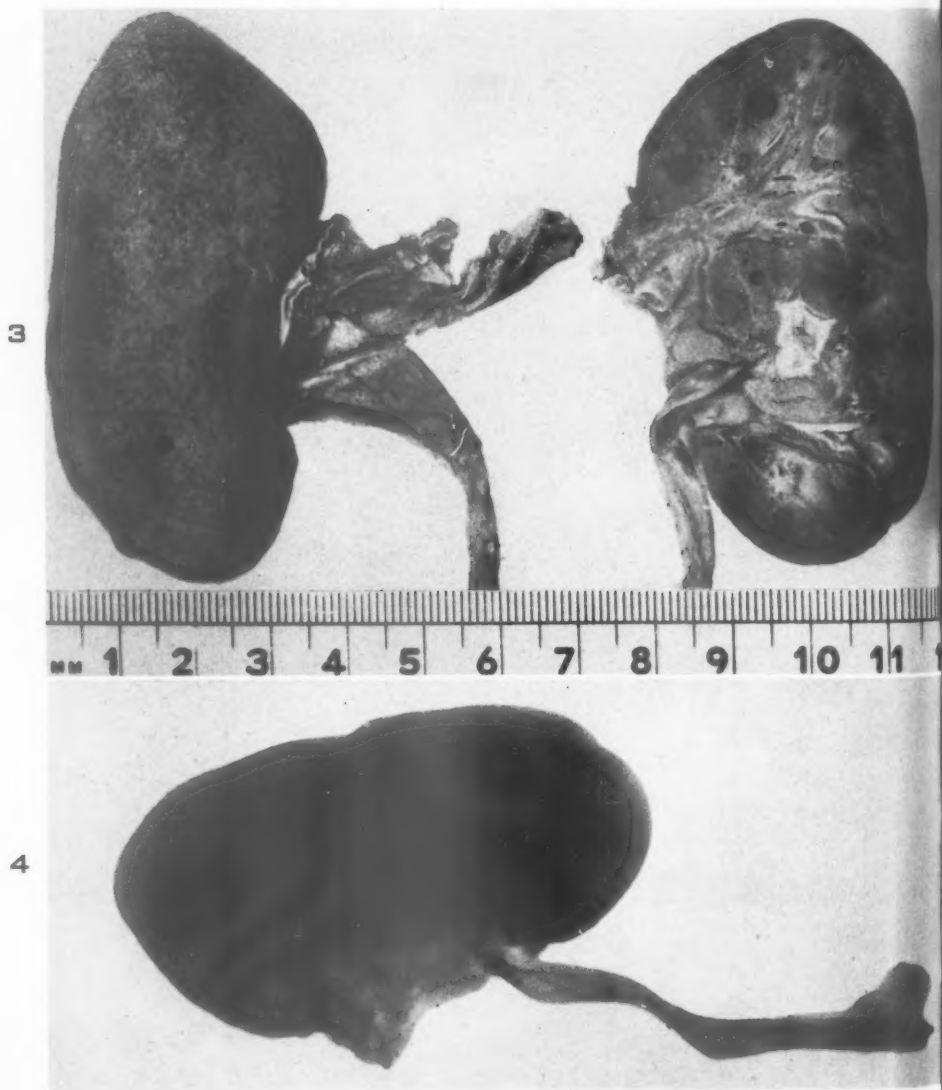
FIG. 4. Case 1. Roentgenogram of kidney showing radiopaque material (calcium and diodone) along the corticomedullary junction.

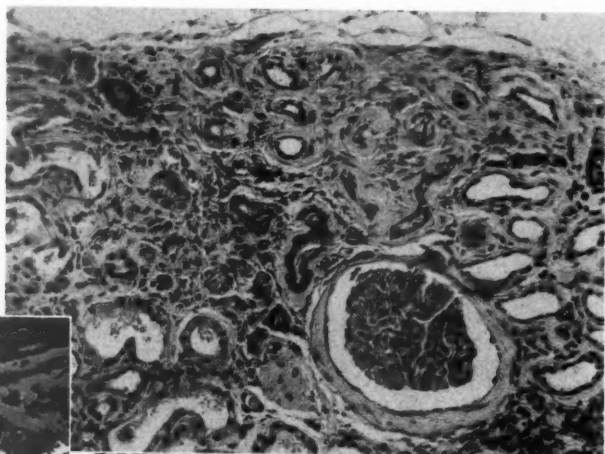
FIG. 5. Case 1. Surface of kidney showing fibrosis. Hematoxylin and eosin stain. $\times 120$.

FIG. 6. Case 1. Crystalline deposit in kidney, half polarized. Hematoxylin and eosin stain. $\times 120$.

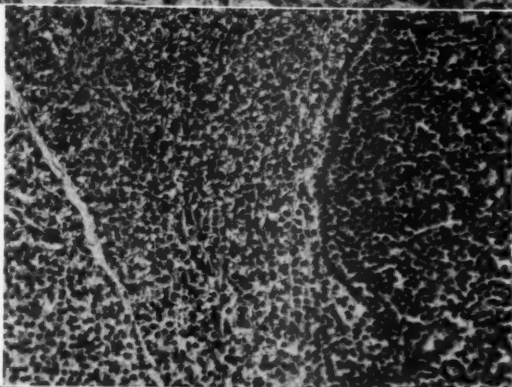
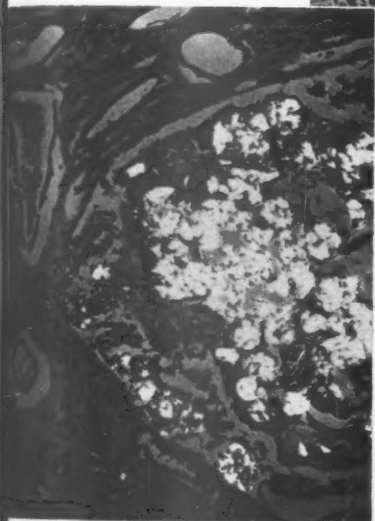
FIG. 7. Case 1. Parathyroid hyperplasia, showing margin of "germinative center." Hematoxylin and eosin stain. $\times 160$.

FIG. 8. Case 1. Parathyroid hyperplasia, showing acinar pattern. Hematoxylin and eosin stain. $\times 150$.

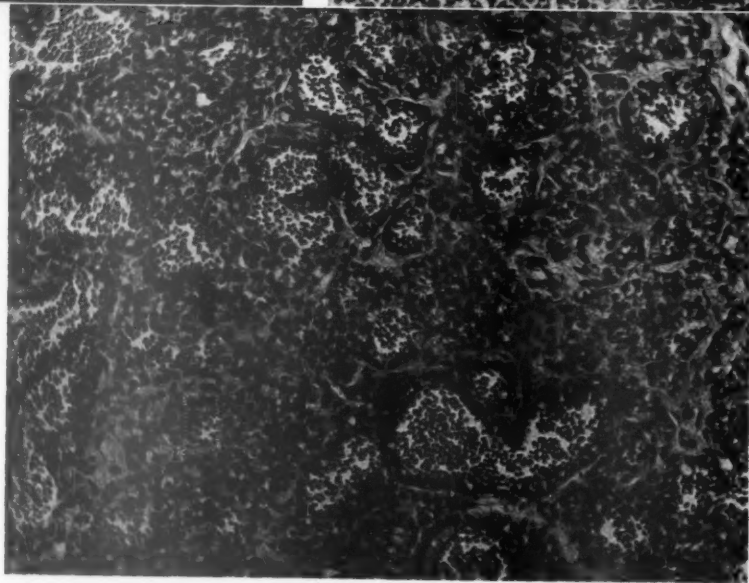




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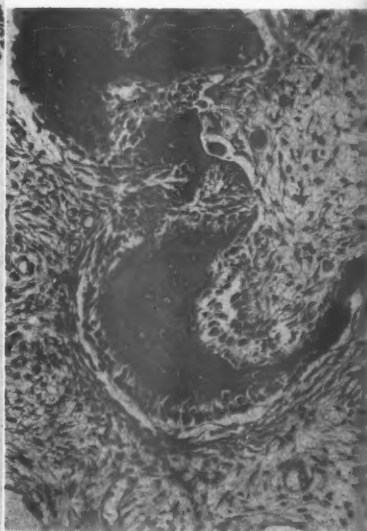
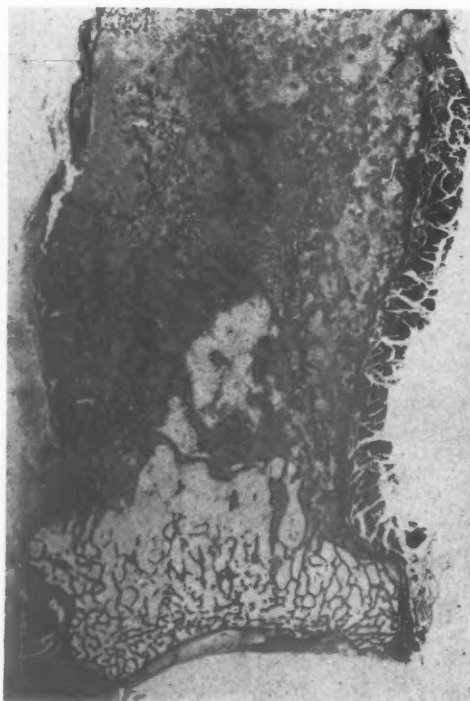
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FIG. 9. Case 1. Lower end of radius, showing osteitis fibrosa. Heidenhain's azan stain. $\times 2.4$.

FIG. 10. Case 1. Osteitis fibrosa, with osteoblastic and osteoclastic activity side by side. Hematoxylin and eosin stain. $\times 110$.

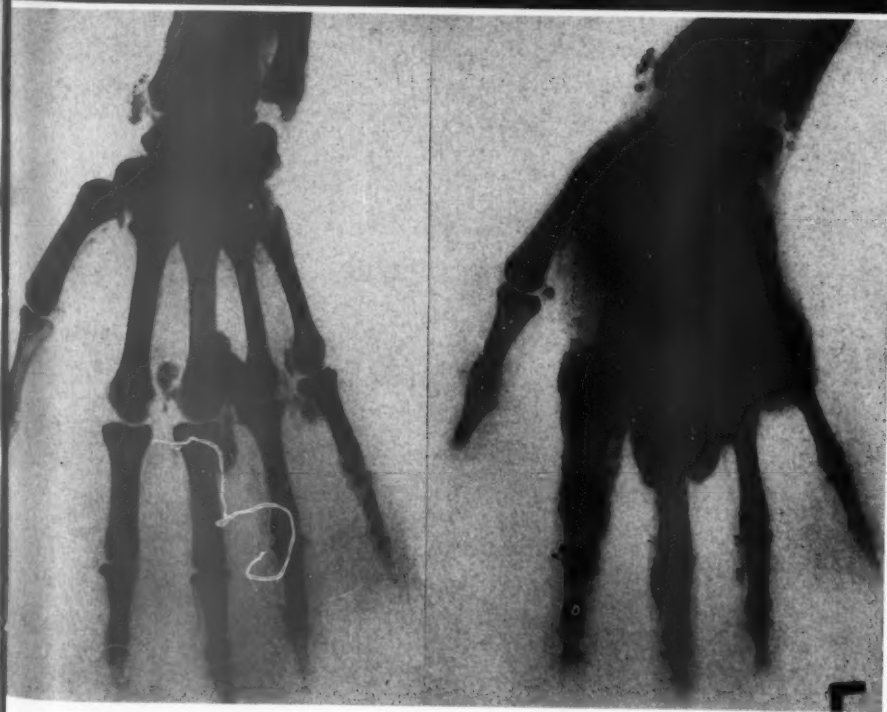
FIG. 11. Case 2. Periarticular swellings of hands.

FIG. 12. Case 2. Roentgenogram of left hand, July, 1942 (left), and January, 1943.





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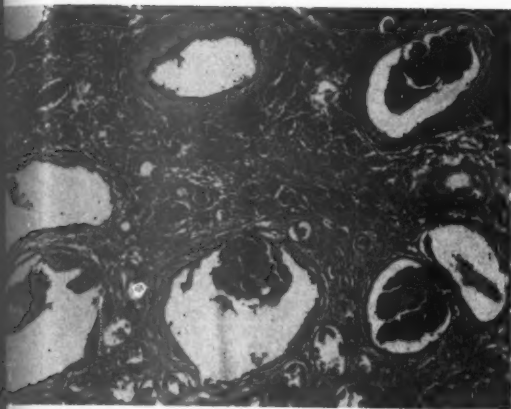
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- FIG. 13. Case 3. Kidney showing wedge of fibrosis, with capsular distention. Hematoxylin and eosin stain. $\times 60$.
- FIG. 14. Case 2. Kidney showing capsular fibrosis and focal calcification. Hematoxylin and eosin stain. $\times 70$.
- FIG. 15. Case 2. Parathyroid hyperplasia showing acinar pattern. Hematoxylin and eosin stain. $\times 100$.
- FIG. 16. Case 3. Primary parathyroid adenoma. Eosinophile nodule compressing original gland. Hematoxylin and eosin stain. $\times 150$.
- FIG. 17. Case 3. Same kidney as shown in Figure 13, showing tubular atrophy and cyst formation. Hematoxylin and eosin stain. $\times 85$.

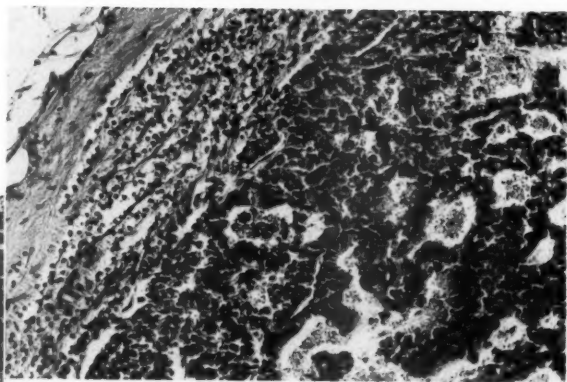
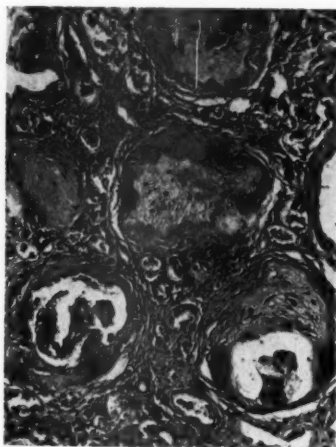
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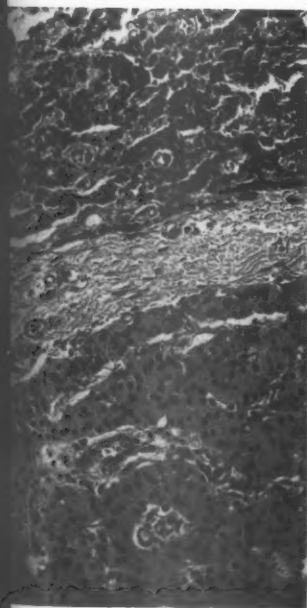
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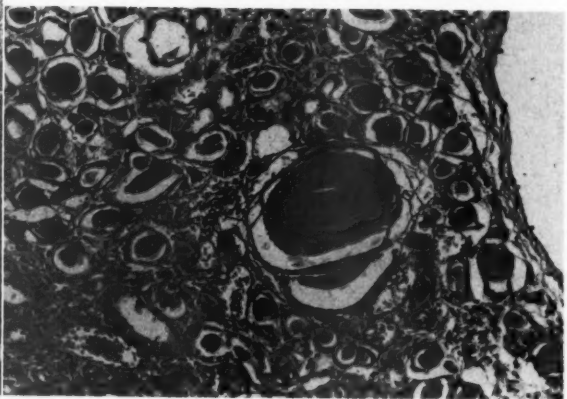
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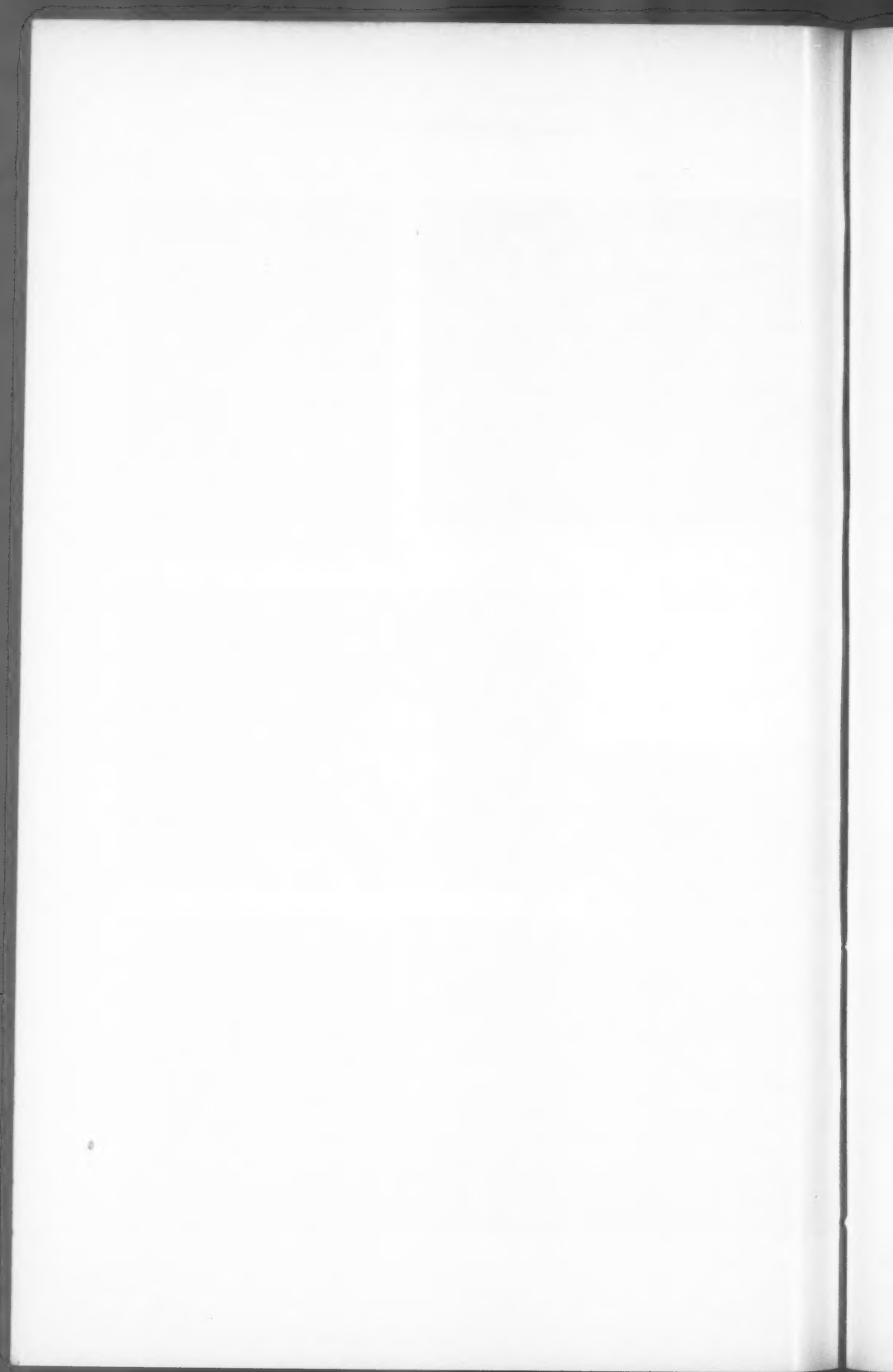
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MYO-EPITHELIUM IN GYNECOMASTIA *

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Langhans,¹ in 1873, first described the existence of myo-epithelial cells in the normal female breast. According to Eggeling,² Günther,³ Hamperl,⁴ Maximow and Bloom,⁵ Smith and Copenhaver,⁶ and others, these cells are found normally beneath the low-columnar epithelium of the lactiferous ducts. They lie within the basement membrane, placed spirally at short intervals with their long axes tangential to the circumference of the duct. The cytoplasm of these cells in most instances is sparse, elongated, and possesses fine fibrillations. When these fibrillated protoplasmic processes extend between and embrace the adjacent epithelium, such myo-epithelial cells are known as basket cells. The nuclei of the myo-epithelial cells vary, being oval, fusiform, or pyriform, and are intensely hyperchromatic. The latter property helps to identify them in routinely stained (hematoxylin-eosin or hemalum-phloxine-saffron) preparations. The most useful stain for demonstration of myo-epithelial cells is Masson's hematoxylin-erythrosin-saffron procedure, which stains their cytoplasm in varying shades of red.

Proliferation of myo-epithelial cells in certain lesions of the human female breast was described in the later years of the nineteenth century by Jüngst⁷ and Dreyfuss,⁸ and more recently by Masson,⁹ Günther,³ Hamperl,⁴ and Kuzma.¹⁰ However, little information is available in the medical literature regarding myo-epithelium in lesions of the male breast. For this reason it was decided to study a series of cases of gynecomastia, in order to determine whether a myo-epithelial component is present in the ductal changes seen in this condition.

MATERIALS AND METHODS

The material for this study consisted of the paraffin blocks obtained from surgical specimens of breast tissue in 20 cases of gynecomastia. Eighteen of these were operated upon in the Ottawa General Hospital in the period from January, 1950, to September, 1953. Two cases came from another hospital. The ages of the patients varied between 13 and 70 years. In none was there evident cause for the gynecomastia such as testicular or pituitary tumor, cirrhosis of the liver, or a history of hormonal therapy. The blocks were re-cut and

* Received for publication, April 12, 1954.

sections were stained by the hemalum-phloxine-saffron and the hematoxylin-erythrosin-saffron procedures of Masson, by van Gieson's stain and by a modification of the silver impregnation method of Bielschowski. However, van Gieson's stain was found to be unreliable for the demonstration of myo-epithelium.

The general microscopic appearance of the breast lesions conformed to Karsner's¹¹ description, and included such features as proliferation of the general stroma and about the ducts, proliferation of ducts with elongation and branching, and varying amounts of lymphocytic infiltration.

PROLIFERATION OF MYO-EPITHELIUM

All the gynecomastic breasts in this series showed some degree of myo-epithelial proliferation, and in several it was marked. Its beginning was manifested by numerical increase of cells, their collection into groups, and change of their usual tangential position into an irregularly radial one. At this stage many myo-epithelial cells were seen lying between the cells of the ordinary ductal epithelium; their nuclei took diverse configurations, being rod-shaped, fusiform, pyriform, or crescentic. One gained the impression that the myo-epithelial cells were trying to squeeze through the overlying epithelium toward the lumen of the duct. The cytoplasm of proliferating myo-epithelial cells sometimes became voluminous and might show either coarse or fine vacuolization. The proliferation of the myo-epithelium might be either pure or accompanied by proliferation of ordinary ductal epithelium and directed either toward or away from the lumen. The latter centrifugal type of proliferation was not common. The rate of myo-epithelial proliferation seemed to be rather slow and mitotic activity was absent. In the cellular groups or masses, during the active stages of myo-epithelial or epi-myo-epithelial proliferation, many cells were encountered, which morphologically could not be classified as either epithelial or myo-epithelial. These cells have been described by Masson,⁹ who termed them *cellules ambigues*.

In pure myo-epithelial proliferation the ordinary duct epithelium appeared to be resting, whereas the myo-epithelial cells showed a tendency to gather into groups. These groups were small at first and might be composed only of a few cells in close apposition, forming bush-like structures protruding into the lumina of the ducts. These myo-epithelial bushes varied in size, might be quite numerous, tended to coalesce, and might line the entire circumference of the duct. Further proliferation of these bushes caused formation of pure myo-epithelial intraductal papillomas and filling of the ductal lumina by irregular cellular masses. Groups of myo-epithelial cells might also be seen

either below the ordinary duct epithelium, which was heaped up by them, or above it as if crowning the latter. This represented a centripetal type of myo-epithelial proliferation, which was common in the cases studied.

The infrequent centrifugal myo-epithelial growth was represented by groups of myo-epithelial cells in the larger mammary ducts, which, while growing, expanded peripherally and pushed the basement membrane ahead of them. Silver impregnation showed some frilling of the basement membrane in these areas, but the membrane still definitely separated the proliferating cells from the surrounding periductal connective tissue. Proliferation of myo-epithelium quite often was accompanied by proliferation of the ordinary duct epithelium, which very frequently was even more pronounced. This proliferation was always centripetal. This mixed proliferation resulted in the formation of epi-my-epithelial papillomas. However, in my cases there was always more myo-epithelium than ordinary ductal epithelium in the mixed papillomas. The myo-epithelium, in spite of its slow rate of proliferation, overgrew and outlived the ordinary epithelium, bringing the process to the same final state—ducts filled with masses of myo-epithelial cells.

REGRESSION OF MYO-EPITHELIUM

In some of these cases remarkable atrophy of the ducts lined by myo-epithelium was noted. This atrophy probably followed either a simple replacement of epithelial lining of the ducts by myo-epithelium without marked previous proliferation, or gradual atrophy of the intraductal masses of myo-epithelium after cessation of previous proliferation. Numbers of collapsed and atrophic ducts were seen within the dense fibrous stroma lined by a single layer of myo-epithelium only, as well as simple linear collections of cells without lumina. The basement membrane around these atrophic ducts and linear collections of myo-epithelial cells was still present but was fuzzy, appeared frilled, and was thicker than usual. Also small irregular myo-epithelial groups incorporated into the fibrous breast stroma resembled former ducts containing either papillomas or solid cellular sheets. These groups were surrounded also by argyrophilic fibers. The myo-epithelial cells in all these groups were mixed with cells which resembled fibrocytes, displayed no fibrillation of their cytoplasm, but had the staining characteristics of myo-epithelium and were surrounded by argyrophilic fibers.

DISCUSSION

Myo-epithelial change in gynecomastia has attracted little attention in the medical literature. In Karsner's¹¹ excellent review of gyneco-

mastia, no mention is made of myo-epithelial proliferation. Weber¹² recognized the existence of myo-epithelium in mammary ducts of male breasts and stated that it is able to withstand pressure exerted upon the ducts by proliferating fibrous stroma longer than the ordinary epithelium. Kuzma,¹⁰ in a paper mainly concerned with myo-epithelial proliferations in the female breast, mentioned that similar proliferations are seen in the ducts of gynecomastia. A study of the material here presented leaves no doubt that there is a large myo-epithelial component in the ductal proliferation encountered in this condition. Proliferation of myo-epithelial cells was seen either alone or with proliferation of the ordinary epithelium of the ducts, usually forming hyperplastic intraductal structures, but occasionally pushing in a centrifugal direction into the surrounding fibrous connective tissue of the breast. Such centrifugal proliferation did not break the basement membrane, but invariably pushed this structure ahead of the resulting cell masses. In no case was the proliferative pattern one which might be confused with malignant neoplastic change.

The part played by myo-epithelial elements in regressive changes in gynecomastic breasts was important. When the ductal elements atrophied, haphazard groupings of myo-epithelial cells, embedded in fibrous tissue but surrounded by basement membrane representing ductal residua, were seen frequently. It appears, then, that in regression of gynecomastic lesions, the myo-epithelial cells of the ducts persist longest but eventually undergo replacement by, or metamorphosis into, fibrocytes and merge into the surrounding stroma.

Little is established regarding the nature of myo-epithelial cells. Morphologically, they resemble smooth muscle cells in certain ways but are located with ordinary epithelium within the basement membrane of ducts. Eggeling² believed that myo-epithelial cells belong to the basement membrane, but Krompecher¹³ and Masson⁹ classified them as epithelial. Masson, as has been mentioned, found certain cells within the lining epithelium of the lactiferous ducts in female breasts which he was unwilling to classify as either epithelial or myo-epithelial and termed them *cellules ambiguës*. It is possible that these *cellules ambiguës* represent a link between epithelium and myo-epithelium in an epi-myo-epithelial metamorphosis. Kuzma¹⁰ wrote that Eggeling² quoted Kölliker as having stated that "myo-epithelium has the faculty of very readily changing into epithelial cells and assuming the cuboidal morphology." However, this statement is not found in the work referred to. On the other hand, Maximow,¹⁴ while experimenting with tissue cultures of rabbit's breasts, noted that the myo-epithelium pos-

sesses a definite tendency to undergo change into fibroblast-like cells. A similar opinion was held by Masson, who described transformation of the myo-epithelium into collagen fibers. Hamperl⁴ traced the origin of mixed tumors of the breasts in dogs to myo-epithelium, and stated that he had seen, in two cases of carcinosarcoma of the human female breast, epithelial cells forming collagen fibers and ground substance. He concluded that there is no sharply defined difference between epithelium and mesenchyme. This may appear strange but there is an analogy with the generally accepted fact that the dilatator pupillae iridis is formed from epithelium (Arey,¹⁵ Maximow and Bloom⁵). According to Hamperl, Maurer¹⁶ suggested the ectodermal origin of the smooth muscle forming the arrectores pilorum and the dartos tunica of the scrotum. From this emerges the rather speculative thought that myo-epithelium may represent a link between ectodermal and mesenchymal elements. Certainly in these gynecomastic breasts there was an indefinite borderline on the one hand between proliferating epithelium and myo-epithelium in hyperplastic intraductal structures and on the other hand between myo-epithelium and fibrocytes in areas showing regressive change.

The function of myo-epithelial cells, like their nature, is still a matter of hypothesis. Maximow and Bloom⁵ and others have suggested that they act in contractile fashion, like smooth muscle, to express secretion from the glands. Drash,¹⁷ according to Hamperl,⁴ claimed to have seen contractions of myo-epithelium when following stimulation of the trigeminal nerve in frogs. Kuzma¹⁰ expressed the thought that they may act as endocrine receptors, transmitting humoral stimuli to the epithelium, and saw no evidence that they are secretory cells. In relation to the latter statement it may be noted that he mentioned vesiculated forms of myo-epithelial cells (unhappily terming them on this account basket cells—*Korbzellen*) without considering the possibility that such vesicles might indicate accumulated secretion. In Hamperl's opinion these vacuoles within the cytoplasm of myo-epithelium represent a degenerative change.

That myo-epithelial cell proliferation plays an important part in the ductal hyperplasia of gynecomastia indicates that such cells, like ordinary ductal epithelium, are influenced by estrogenic hormones, since it is generally believed that relative or absolute increase in estrogen forms the basis of this condition. However, this study gives no clue as to the function of myo-epithelium in health or in disease. Nevertheless, there are morphologic indications that ordinary ductal epithelium may undergo myo-epithelial metamorphosis with the *cellules ambiguës*

of Masson representing an intermediate link in this myoid metaplasia. It seems also, that in regressive lesions a fibrocytic metamorphosis may take place. This morphologic variability suggests the existence of a similar functional versatility. It is possible that myo-epithelial cells, depending on their state and situation, may act as either secreting or contractile cells, subject to endocrine stimuli.

Hamperl⁴ and Kuzma¹⁰ have stressed that in cystic mastopathia in the female myo-epithelial proliferations have been confused with malignant change or have been given a precancerous significance. They indicated that buds of myo-epithelial cells proliferating into stroma or nests of myo-epithelial cells which have survived in areas of hyaline fibrosis may suggest malignant neoplasia. Non-neoplastic intraductal myo-epithelial proliferative lesions may be interpreted as malignant transformation in ordinary intraductal papillomas. While the possibility of the development of changes leading to errors of this type may exist, in the cases of gynecomastia studied no such lesions were seen. In areas of regression in gynecomastic breasts the presence of groups of irregular, densely-staining, myo-epithelial nuclei embedded in dense fibrous tissue, might puzzle those unfamiliar with their nature, but would be unlikely to be interpreted as cancer.

SUMMARY

Proliferation of myo-epithelium plays an important part in the ductal hyperplasia of gynecomastia. Myo-epithelial cells may proliferate either alone or together with the ordinary ductal epithelium to form benign intraductal papillomatous growths. Proliferating myo-epithelial cells may also extend into the periductal connective tissue, forming benign outgrowths still confined by the basement membrane. When regression with ductal atrophy occurs in gynecomastia, the myo-epithelial cells persist after the ordinary epithelial cells have vanished and often remain as bizarre cell groupings embedded in dense fibrous tissue. None of these types of myo-epithelial activity is likely to be confused with changes of a malignant neoplastic nature.

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[Illustrations follow]

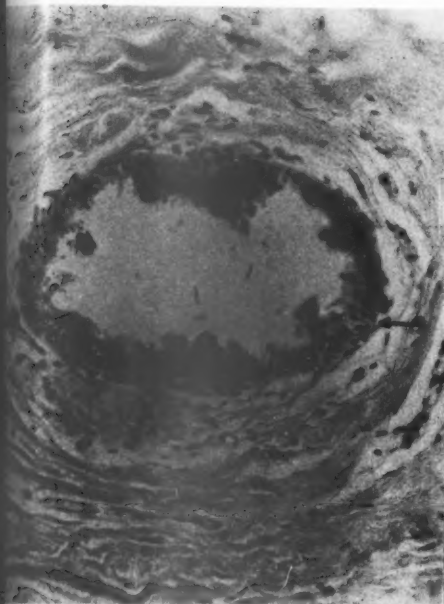
LEGENDS FOR FIGURES

- FIG. 1. Gynecomastia. A duct showing myo-epithelial cells (indicated by arrow) lying tangentially beneath the ordinary epithelium. Along the circumference of the duct "bushes" of proliferating myo-epithelium are seen. Hemalum-phloxine-saffron stain. $\times 415$.
- FIG. 2. Gynecomastia. A segment of the duct shown in Figure 1, from the area marked by the arrow. Four myo-epithelial cells lie in their normal positions between the ordinary duct epithelium and the basement membrane. Another myo-epithelial cell with a dark rod-shaped nucleus is lying radially, between ordinary epithelial cells. Hemalum-phloxine-saffron stain. $\times 1010$.
- FIG. 3. Gynecomastia. A duct showing myo-epithelial cells proliferating in bush-like fashion. Many myo-epithelial cells are vacuolated. Hematoxylin-erythrosin-saffron stain. $\times 415$.
- FIG. 4. Gynecomastia. Small ducts showing pronounced proliferation of columnar epithelium. The darker, slender, myo-epithelial cells are seen between columnar epithelial cells, thrusting toward the lumina of ducts. The arrows indicate "crowning" of the columnar epithelium by myo-epithelium. Hematoxylin-erythrosin-saffron stain. $\times 415$.

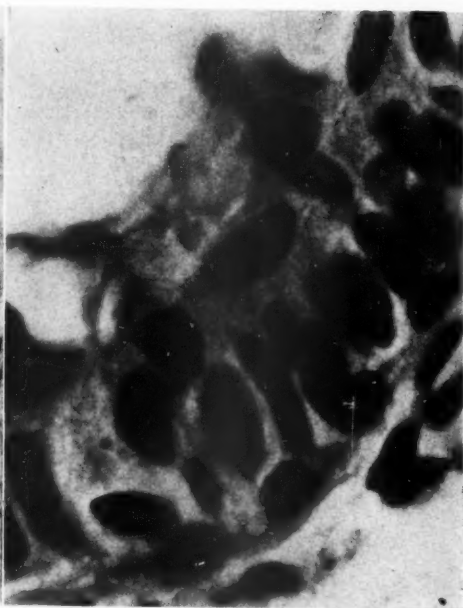
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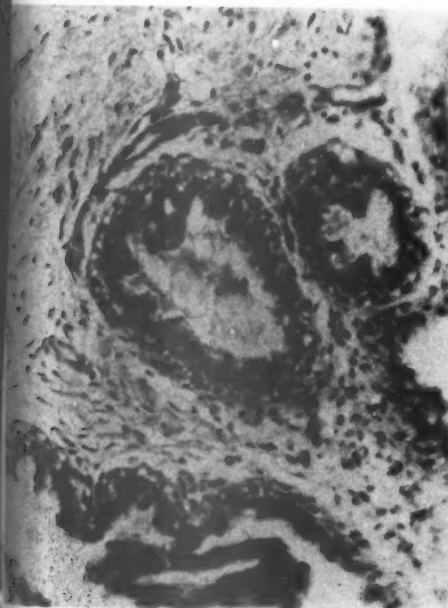
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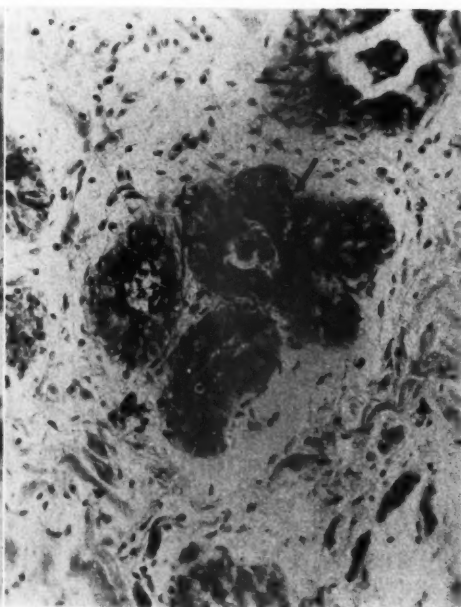
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FIG. 5. Gynecomastia. A duct lined by low columnar epithelium contains a broad-based papillomatous growth composed entirely of myo-epithelium. Hematoxylin-erythrosin-saffron stain. $\times 415$.

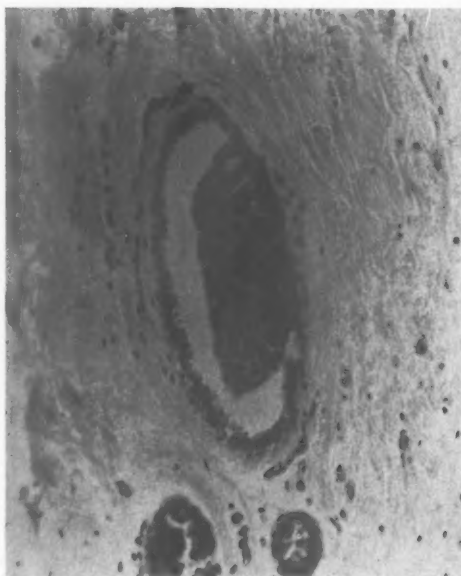
FIG. 6. Gynecomastia. Two ducts containing mixed epi-myo-epithelial papillomas. Hematoxylin-erythrosin-saffron stain. $\times 210$.

FIG. 7. Gynecomastia. A large duct shows scattered myoepithelial "bushes" and areas of myo-epithelial proliferation directed into fibrous stroma. Hematoxylin-erythrosin-saffron stain. $\times 210$.

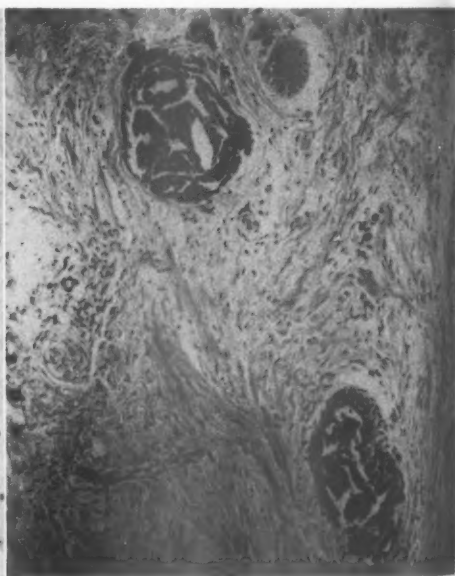
FIG. 8. Gynecomastia. A large duct lying within fibro-fatty tissue is packed by masses of myo-epithelium. Hematoxylin-erythrosin-saffron stain. $\times 210$.

FIG. 9. Gynecomastia. Atrophic ducts reduced to clusters and linear streaks of myo-epithelium. Hematoxylin-erythrosin-saffron stain. $\times 210$.

FIG. 10. Gynecomastia. Atrophic duct lined by myo-epithelium and surrounded by a thick and frilled basement membrane. At one side of the duct a cluster of myo-epithelial cells is seen. This cluster is surrounded by disintegrating basement membrane. Hematoxylin-erythrosin-saffron stain. $\times 595$.



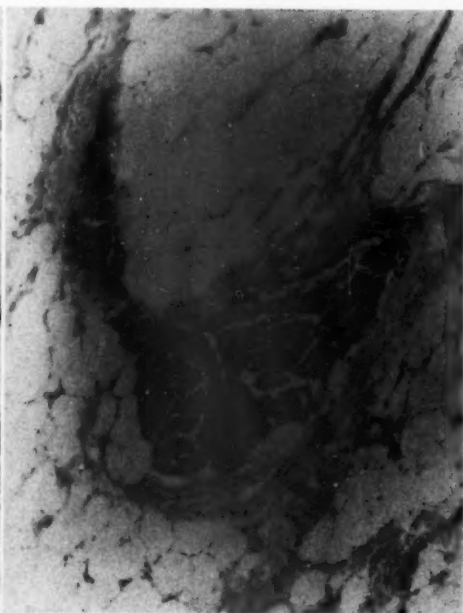
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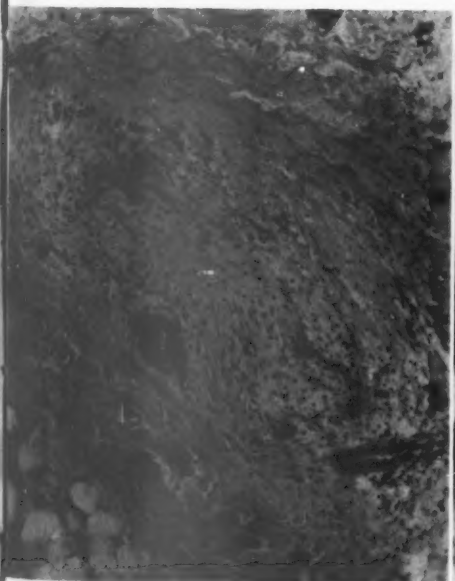
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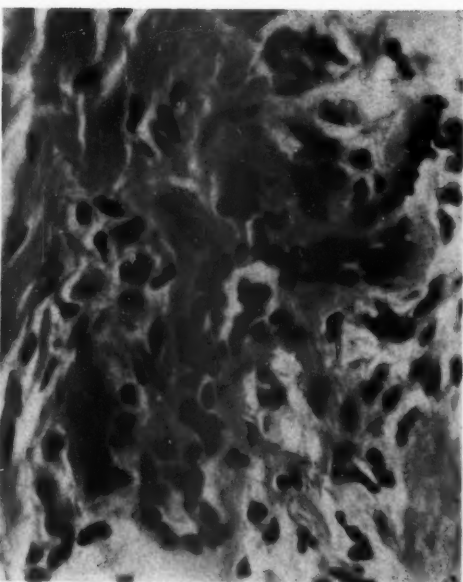
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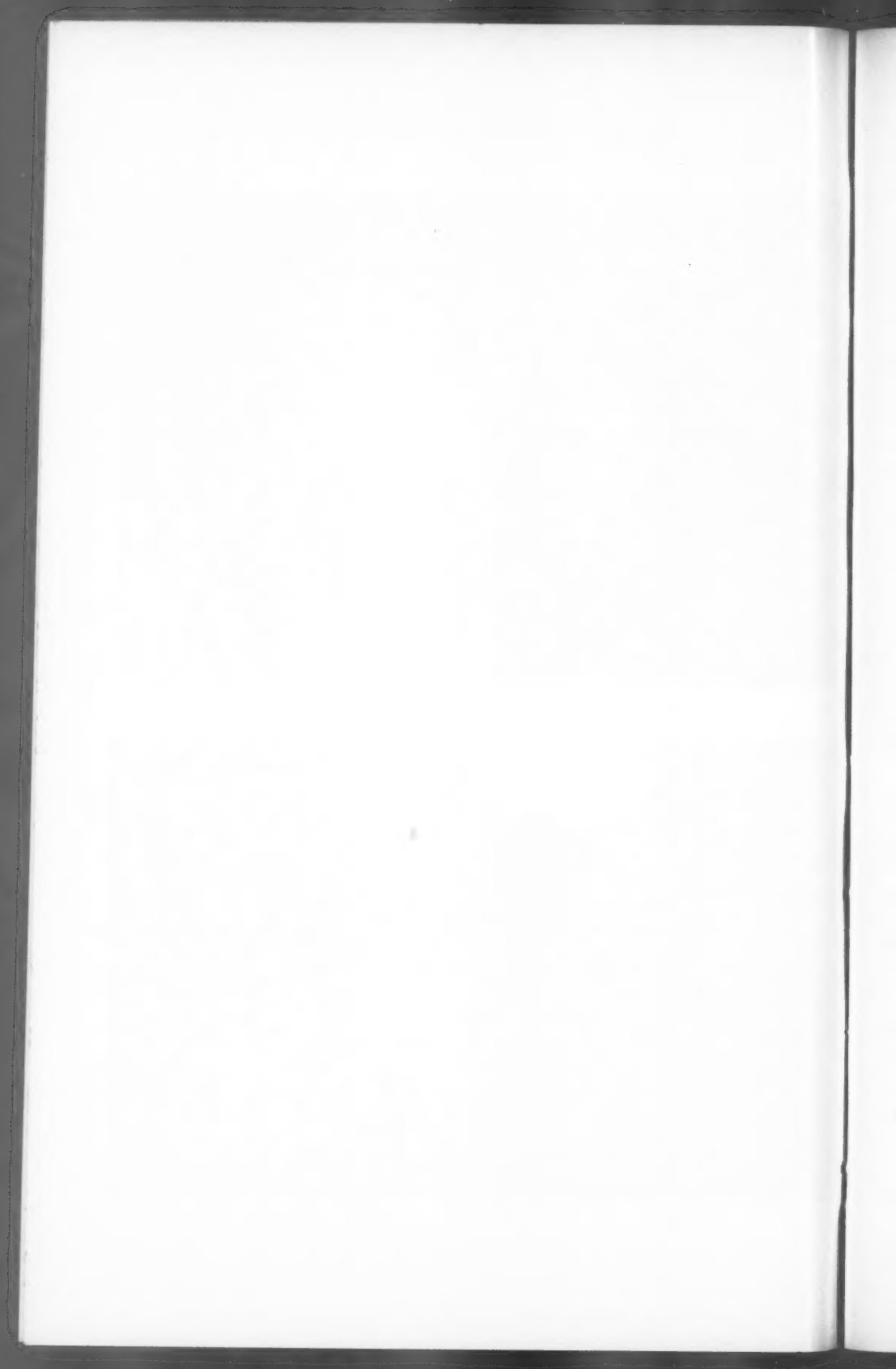
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A MICROSPECTROSCOPIC STUDY OF ARTERIOLES IN BENIGN AND MALIGNANT HYPERTENSION *

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Various histochemical procedures applied to normal human and canine arterioles, acutely necrotic arterioles of the dog following bilateral nephrectomy, acutely necrotic arterioles of man in malignant hypertension, and hyalinized arterioles of man associated with benign hypertension have yielded similar results for the area of the media.^{1,2} These observations were considered to support the previous stand, based on transitions detected by less precise methods of staining,³⁻⁷ that the change within the arterioles in these various conditions is the result of injury and subsequent alterations of the smooth muscle of the media. In these discussions³⁻⁶ it was appreciated that other changes contributed to the ultimate lesion of arteriolar sclerosis, as for example the deposition of connective tissue elements within the various layers of the vessel wall, but the fundamental element in pathogenesis was considered to be injury and alteration of smooth muscle. The purpose of the present paper is to present data obtained by microspectroscopic analyses of the same lesions studied histochemically in two previous reports,^{1,2} which can be considered to give further support to the thesis that the hyalin of hyaline arteriolar sclerosis is derived mainly from the fusion of degraded products of smooth muscle of the media.

METHODS

The material from man and the dog considered herein was that previously studied histochemically.^{1,2} The human material consisted of the lesion of acute arteriolar necrosis in the kidney of a patient dying with classical malignant hypertension and of those of hyaline arteriolar sclerosis in the kidneys of 4 necropsied patients with classical benign hypertension. One of these had severe diabetes with nodular glomerular sclerosis. The normal or control human arteriole was obtained from the periadrenal fat of a young adult who died from trauma. The example of acute arteriolar necrosis of the dog was obtained from a hypertensive bilaterally nephrectomized dog maintained as previously

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described.⁷ The normal canine arteriole was obtained by the sacrifice of a healthy adult mongrel dog.

In each instance, one arteriole was studied in one frozen section of formalin-fixed tissue. The frozen sections were cut at approximately $6\ \mu$ and mounted unstained in glycerin on Vycor* slides with Vycor* coverslips. The arteriole to be studied was selected and brought into focus with visible light. By means of a grating monochromator† with a hydrogen arc source and reflecting quartz optics,‡ monochromatic light at intervals of $5\ m\mu$ from 250 to $360\ m\mu$ was passed through the tissue section and the image recorded on S. A. No. 1 film§ held in a 35 mm. gamma camera back. The exposure time for each wave length differed and was predetermined with a blank slide so that a constant background density of the film was obtained at all wave lengths. The photographs thus obtained represent comparative absorptions at various intervals and may be compared quantitatively for any one lesion. Since no effort was made to control accurately the thickness of the preparation or the distribution of the material within the lesion, one lesion could not be compared quantitatively with another. However, the lesions could be compared qualitatively from the curves obtained by plotting the direct densitometric readings of the image as recorded by the film against the wave lengths of light. To obtain these readings the image of the lesion on the film at each wave length was masked and placed in front of a densitometer§ head while a constant source of visible light was passed through the film to the densitometer. Variations in the densitometer readings at various wave lengths thus represented variations in the absorption characteristics of the material photographed. By comparing the shapes of all the curves so obtained one could appraise the nature of the absorbing substances in each case.

RESULTS

Text-figure 1 is a composite graph of the curves of the normal and abnormal human and canine arterioles. The curves represent the film densities plotted against wave length in millimicrons. These curves show similar absorption patterns, which indicate a very high absorption from 250 to $290\ m\mu$. At the latter wave length all of the curves rise, indicating less absorption of the light at subsequently higher wave lengths. The height of the rise is not the same in all cases, although the

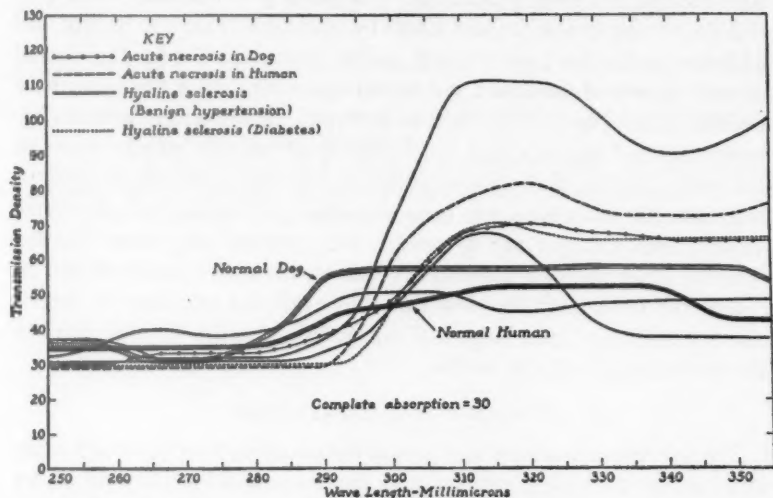
* Vycor slides and coverslips from A. D. Jones Optical Company.

† Bausch & Lomb reflecting optics, grating monochromator with hydrogen arc light source.

‡ S. A. No. 1 film from Eastman Kodak Company.

§ Photovolt densitometer, Model 520M.

curves show a similar distribution from 290 to 350 $m\mu$. These data indicate that the absorption characteristics of normal human and canine arterioles, acutely necrotic human and canine arterioles, and hyaline human arterioles are similar from 250 to 350 $m\mu$.



Text-fig. 1. Microspectroscopic absorption curves for wave lengths from 250 to 350 $m\mu$ for normal human and dog arterioles, acutely necrotic human and dog arterioles, and hyaline human arterioles.

In the normal and the necrotic arteriole the main tissue concerned in the absorption pattern is the smooth muscle of the media. The finding that the hyalinized arteriole of arteriolar sclerosis has the same absorption characteristics as its necrotic counterpart is considered an additional indication of origin of the hyaline material from smooth muscle. This is demonstrated by Figures 1 to 6, which represent the absorption photographs of a hyalinized arteriole from case 4 of Montgomery and Muirhead² at 255, 285, 290, 295, and 345 $m\mu$ and visible light, respectively. Normal vessels, vessels with acute necrosis, and hyalinized vessels show essentially the same photographic pattern as indicated by the absorption curves reproduced in Text-figure 1.

The precise chemical nature of the absorbing substances in each of the cases cannot be determined by an analysis of these absorption curves. Normal and abnormal arterioles are known to contain a wide variety of substances, demonstrable by histochemical means, including lipids, carbohydrates, free carbonyl groups, protein-bound sulfhydryl groups, and free potassium; the normal vessels and the acute necrotic

arterioles contain acid phosphatase in addition, while the hyaline arterioles have not given this enzymatic reaction.^{1,2}

In this institution, normal arterioles, necrotic arterioles, and hyalinized and sclerotic arterioles have been studied by a battery of conventional staining procedures,³⁻⁷ a battery of histochemical procedures,^{1,2} and in the present study by microspectroscopic means. All of these approaches have yielded similar characteristics for the normal smooth muscle of the media, the acutely necrotic media, the subacutely necrotic media, and the hyalinized arteriole. Moreover, transitions between isolated necrosis and hyalinization of smooth muscle fibers of the media, fusion of these altered fibers, and the ultimately hyalinized and sclerotic arteriole have been repeatedly detected and described. Transitional changes are by nature less precise, but, when coupled with the other more objective observations, the entire spectrum can be considered to support the view that the hyalinized substance of arteriolar sclerosis is at least in part, if not mainly, derived from changes in the smooth muscle of the media.

SUMMARY AND CONCLUSIONS

The microspectroscopic absorption characteristics of normal human and canine arterioles, human and canine arterioles showing acute arteriolar necrosis, and human arterioles showing hyaline sclerosis were determined and compared by direct film densitometry.

The absorption curves of these normal and abnormal arterioles show complete absorption from 250 to 290 μ . From 290 to 350 μ the curves show incomplete absorption and are qualitatively similar.

These data support the view that the acute arteriolar necrosis of the bilaterally nephrectomized dog, the acute arteriolar necrosis of human malignant hypertension, and the hyaline sclerosis of human benign hypertension have a common pathogenesis related to alterations of the smooth muscle of the arteriolar media.

We wish to thank Mrs. Ovia Walker for the photographs which appear in this article and for her technical assistance.

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[Illustrations follow]

LEGENDS FOR FIGURES

All illustrations show the same hyaline arteriole from case 4 of Montgomery and Muirhead,² in unstained formalin-fixed frozen sections. Magnification, 53 X. Enlargement, 150 X. Wave length used as indicated in the individual legends.

FIG. 1. 255 millimicrons.

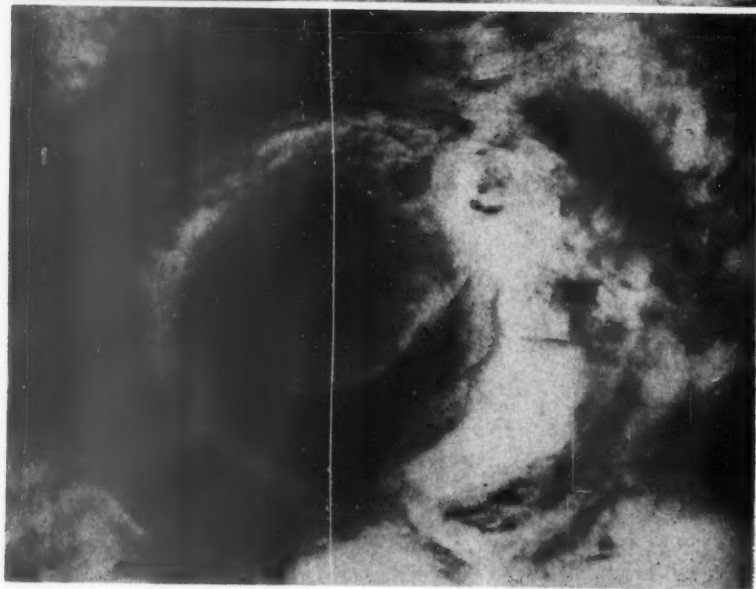
FIG. 2. 285 millimicrons.

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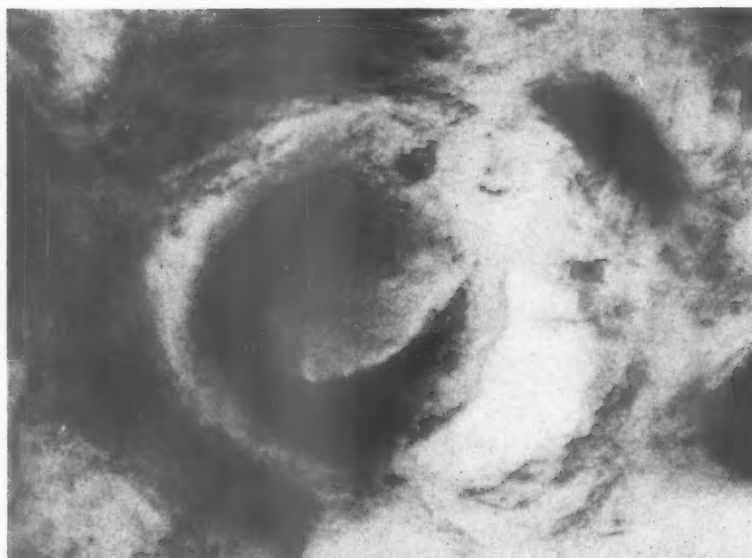


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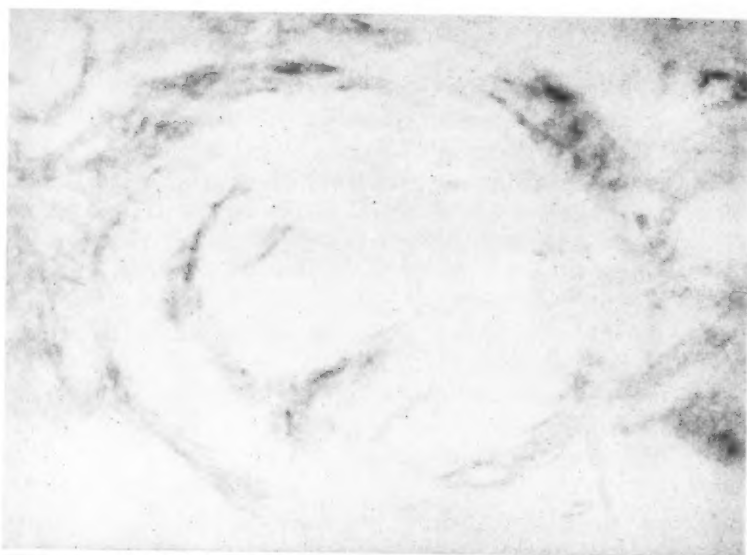


FIG. 3. 290 millimicrons.

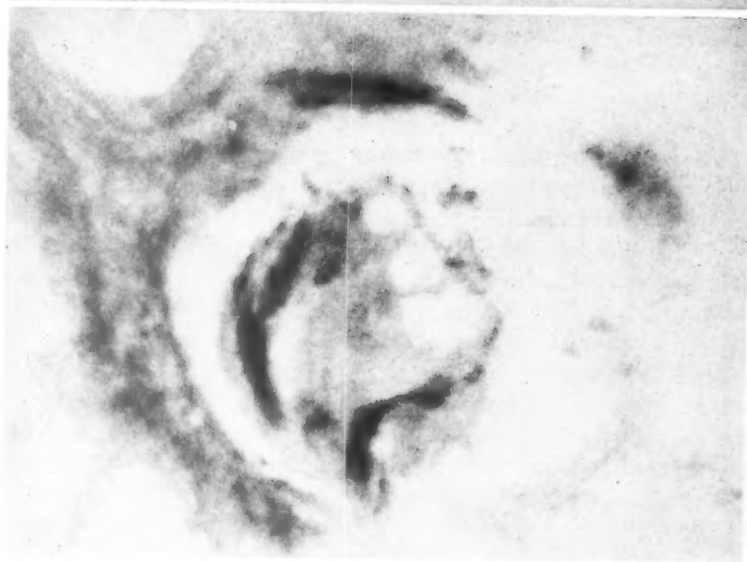
FIG. 4. 295 millimicrons.

FIG. 5. 345 millimicrons.

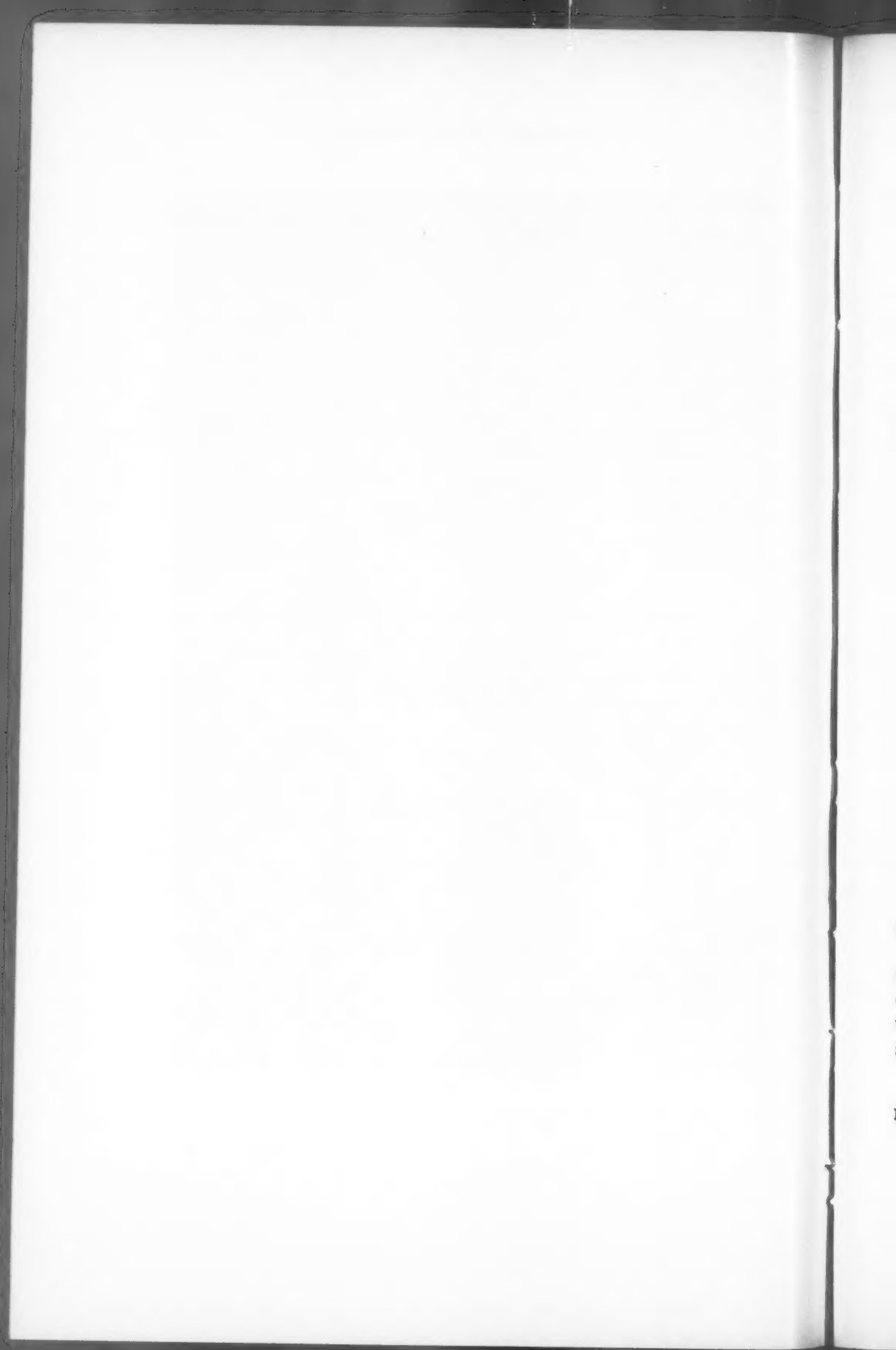
FIG. 6. Visible light.



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THE ORIGIN OF SACROCCYGEAL PILONIDAL SINUSES
BASED ON AN ANALYSIS OF FOUR HUNDRED SIXTY-THREE CASES*

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Pilonidal sinuses, with associated cysts or abscesses, have been recognized for over 100 years; however, the recent war served to make physicians more aware of the prevalence and the importance of this condition. The articles written during the war years (1942-48) nearly equalled in number all that had appeared previously. Most of these papers were concerned with treatment and presented conflicting opinions as to the proper surgical procedure. This, however, has not been the only area of confusion. There has been no unanimity of opinion as to origin and pathogenesis. Most writers have agreed that these lesions arise upon a congenital basis but have disagreed upon the pathogenesis. Recent reports of similar sinuses in other regions have revived interest in earlier suggestions of an acquired rather than a congenital origin for sacroccygeal pilonidal sinuses.

In 1946 and 1948 Patey and Scarff^{1,2} described a lesion on a barber's hand with histopathologic findings identical with those of a pilonidal sinus. They concluded that pilonidal sinuses could not be explained fully on the usually accepted developmental basis but considered them to be acquired infective and foreign body granulomatous reactions to buried hair. In 1947, King³ also suggested that pilonidal sinuses and cysts were results of infection and foreign body granulomatous reaction to hairs which were introduced into the sinuses from without. The example recorded by Ewing⁴ in the same year was less convincing to that author. Recently, Downing⁵ has described another example of pilonidal sinus of the hand in a barber who had expressed hair shafts repeatedly from a lesion of the right second interdigital space, an area in which hair follicles do not occur normally. Apparently, interdigital pilonidal sinus may be considered an occupational disease of barbers.

Examination of such reports of pilonidal lesions in other than the usual area led me to reconsider the problem of the pathogenesis of this condition. There was a significant implication that the essential basis was acquired rather than developmental. Accordingly, a comprehensive review of the literature as to pathogenesis was undertaken and

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pertinent information was extracted from an unselected series of 463 cases on which microscopic sections were available.

HISTORICAL REVIEW WITH REFERENCE TO ORIGIN AND PATHOGENESIS

Anderson,⁶ in 1847, apparently was the first to describe the condition now known as pilonidal sinus, in a report on *Hair Extracted from an Ulcer*. In 1854, Warren⁷ gave a good description of such lesions and reported 2 cases. Later,⁸ adding another case, he suggested a theory as to origin and a method of treatment. He believed that the condition began in a single follicle from a hair which became inverted and continued to grow, forming a tangled mass. Constant pressure and moisture of the area "softened both the newly formed hair and epidermis cells surrounding the mouth of the follicle."

Hodges⁹ was one of the first to consider a congenital basis in the pathogenesis of pilonidal sinuses. He believed three factors to be essential to their development: a congenital coccygeal dimple, abundant development of hair in the area, and poor local hygiene.

Lannelongue¹⁰ demonstrated that the skin over the coccyx is tightly adherent in the embryo, and that, as the surrounding mesoblastic structures increase in thickness, the natural result would be for the skin to be pulled in at this point, forming a dimple. Any exaggeration of this dimple would cause the formation of a sinus. Then, if the epithelium over the external opening proliferated, it would occlude the mouth, forming a cyst. Since epidermal epithelium lined the cyst, normal skin appendages could be found in the cyst.

Tourneux and Herrmann¹¹ (1887) believed that the embryonic neural canal remains attached to the skin. As the fetus develops, the spine grows more rapidly than the surrounding tissues, and, as a result, the end pulls on the skin, forming a U-shaped epithelial cord around the tip of the coccyx. Normally, this portion of the cord atrophies and disappears after the fifth fetal month; however, occasionally it remains. Since it is composed of stratified squamous epithelium, it may develop a cyst or sinus containing the accessory dermal structures such as hairs and sebaceous and sweat glands.

A few years earlier, Féré¹² had stated, without elaboration, that pilonidal sinuses were slight defects in the coalescence of the superficial portions of the medullary folds in the sacrococcygeal region. This theory, which explained pilonidal sinuses on the basis of incomplete fusion of the lateral halves of the dorsum of the fetus, gained numerous adherents, including Sutton.¹³

These basic hypotheses have been supported and somewhat modified by subsequent workers. Mallory¹⁴ examined the caudal end of seven

fetuses, 3 to 6 months old, and concluded that obliteration of the medullary canal takes place first and most completely at the level of the lower end of the sacrum and extends from this point in both directions. He thought that pilonidal sinuses probably originated through incomplete obliteration of a previously existing canal; and, since these lesions extend upward and posteriorly, the medullary canal seemed the most likely place of origin.

Bookman¹⁵ concluded that pilonidal sinuses were due to displacement of "dermal or dermoid" cells in the embryo during the process of fusion in the midline, with these cells assuming an abnormal location under the skin. Collection of epidermal products, as hair and sebaceous material, results in the formation of a cyst.

Stone^{16,17} stated that pilonidal sinuses must be regarded as special downgrowths of epithelium originating from the skin. He suggested that they may be a phylogenetic representation of the preen gland which is found in some species of birds. After detailed study of the human embryo, Fox¹⁸ concluded that pilonidal sinuses are derivatives of cutaneous ectoderm which has persisted after the period of normal ectodermal invagination has been completed. He stated that an analogy can be drawn, because of this mode of origin, between pilonidal sinuses and a special "scent" gland in the sacrococcygeal region of birds and amniotes. To him this suggested that they might represent a vestigial skin appendage developing at puberty, thus explaining the age incidence of the lesion. Somewhat along a similar vein were the conclusions of Kallet.¹⁹ He insisted that the concept that in the sacrococcygeal region there are present at birth embryonic remnants of a vestigial secondary sex gland which is activated at adolescence by the pituitary body better explains the clinical picture than the previously mentioned points of view.

Of more recent writers, Gage²⁰ was the chief proponent of the "neurogenic" theory, *i.e.*, that pilonidal sinuses are due to persistence of the neurenteric or neural canal. According to this view, normally, in the caudal end of the embryo, the portion of the neural canal that is formed by the neural folds and lies between the skin and the coccygeal vertebrae is obliterated by cohesion of its walls. Failure to do so forms a cavity which may be connected to the skin by one or more sinuses. The lining of the cavity retains its capacity to produce primitive skin appendages and a rudimentary type of nervous tissue, if a connection with the spinal canal remains. He believed that the sacral dimple is the result of an anterior pull on the overlying skin by the caudal ligament as the coccyx grows downward and curves anteriorly. This latter idea on the origin of the sacral dimple was first described

by Oehlecker²¹ when he called attention to the relative absence of hair in the area, naming it the "sacral bald spot."

Most subsequent workers are of the opinion that pilonidal sinuses are congenital in origin, and are proponents of either the ectodermal invagination theory or the neurogenic theory.

It was not until 1880 that the name "pilonidal" (pilus: a hair + nidus: a nest) was given to the lesion by Hodges.⁹ Since then a variety of names have been applied. Some of the more common names are: sacral, coccygeal or sacrococcygeal infundibulum; dermoid, and dermoid fistula, sinus, or cyst; posterior umbilicus; post-anal dermoid; congenital dermal sinus, and sacrococcygeal ectodermal sinus. However, pilonidal sinus is now the term most generally used.

STRUCTURAL FEATURES OF SIGNIFICANCE IN PATHOGENESIS

The sacrococcygeal pilonidal sinus consists of an acute or chronic inflammation of a localized area. A small opening is seen usually in the midline about 3.5 to 5 cm. posterior to the anal orifice, between, and usually concealed by, the buttocks. This opening, from which hairs may protrude, leads into an epithelium-lined sinus which is directed upward. There may be a bulbous ending to this single tract; or there may be a number of sacculations communicating with each other by way of the main tract or lateral tracts; or some of these may communicate with the skin through other orifices. These sacculations vary in size and are usually filled with vascular pyogenic granulation tissue.

By 1946, sixteen lesions resembling pilonidal sinuses had been reported which had dural connections in direct continuity with the filum terminale.²² Two of these were associated with spina bifida occulta,^{23,24} and two were associated with meningitis.^{25,26} These cases are believed to be examples of congenital anomalies and not of true pilonidal sinuses. If Gage²⁰ correctly recognized glial tissue beneath the epithelial lining, his case, also, must have been a congenital anomaly.

The presence of cutaneous appendages, as hair follicles, sebaceous glands and sweat glands (described first by Gussenbauer²⁷ in 1893, and by Crone²⁸ in 1917), as part of the epithelial lining has been asserted by various writers including Stone^{16,17} and Rogers and Hall.²⁹ Others have not been able to confirm these findings.^{1,3,21,30} They have found areas of acute or chronic inflammation with numerous foreign body giant cells, some hairs without hair follicles, small islands of squamous cells either free or attached to the lining of the sinus, and squamous epithelium at the entrance of the sinuses. Oehlecker²¹ made careful microscopic studies (including serial sections) but found no epithelial structures. It is difficult to explain the conflict of opinions on the pres-

ence of hair follicles and other accessory skin structures. This is a matter of objective observation and should have only one answer. Does it mean that those who claimed the presence of such structures attributed to sinus epithelium and wall, structures which actually pertained to the surface epidermis?

INCIDENCE

Pilonidal sinuses are much more common than was supposed by earlier workers. This lesion is most common in early adult life—most reported cases occurring between the ages of 16 and 25 years.^{18,19,30-32} It was once thought that the condition was rare in women³³; but, recent studies have shown this is not the case.^{18,19,30-32} However, in most series the incidence in males is greater than that in females.³⁴⁻³⁷

It was not until 1934³⁸ that a case was reported in a Negro. It had been believed that pilonidal sinus was a condition confined exclusively to the Caucasian race.³⁹ In 1935, Breidenbach and Wilson³⁰ reported 4 cases in Negroes in a series of 288 cases from Bellevue Hospital in New York City. By 1947, 21 cases had been reported.³⁶ It is interesting to note that in these reports the incidence is higher in females.³⁴

The occurrence of sacrococcygeal sinuses in identical twins has been reported by Goldberg and Bloomenthal,³³ Mechling,⁴⁰ and Fox.⁴¹ A familial incidence has been mentioned by Tendler^{36,42} and others.^{34,35}

ASSOCIATED ETIOLOGIC FACTORS

Many factors have been found to be more or less constant in cases of pilonidal sinus and have thus been designated as associated etiologic factors, or contributing causes. Two of these, hirsutism and poor local hygiene, were mentioned as early as 1880 by Hodges.⁹ Later papers emphasized these and other factors, such as trauma and obesity.^{2,3,35,43-47} Kallet,¹⁹ in 1936, stressed adolescence as a factor associated with the development of pilonidal sinuses.

MATERIALS

A study series of 463 cases of pilonidal sinus was derived from the files of the Department of Pathology of the University of Michigan. Only the cases in certain years were used, but within those years the cases were unselected. All lesions were sacrococcygeal. The University Student Health Service supplied many cases; others were from University Hospital and various outside hospitals submitting surgical material for diagnosis. Pilonidal sinuses occurred in a ratio of approximately 1:500 specimens from other surgical procedures.

The few cases which had been diagnosed as congenital dermoid cysts, epidermoid cysts, and meningocele were excluded. The micro-

scopic sections from all cases were re-examined, and all gross material submitted with a clinical diagnosis of pilonidal sinus during a period of 7 months was examined carefully as it was sectioned.

RESULTS

Incidence. Since the cases were unselected and consecutive within the years used, clinical data were often incomplete. As to incidence in respect to sex, there were almost exactly twice as many males (304) as females (149) among those of a stated sex. This difference is probably unfairly weighted in favor of males because of male preponderance in the student body from which numerous cases were derived.

The age distribution by quinquennia, expressed in case units and also as a percentage of those of stated age, is shown in Table I. In

TABLE I
Over-all Age Incidence

Age in years	No. of cases	Per cent of total	Age in years	No. of cases	Per cent of total
0-5	3	0.9	36-40	16	5.0
6-10	0	0.0	41-45	7	2.2
11-15	4	1.3	46-50	2	0.6
16-20	87	27.4	51-55	3	0.9
21-25	110	34.6	56-60	3	0.9
26-30	43	15.1	61-65	1	0.3
31-35	32	10.1	66-70	2	0.6
			Total	318*	100.0

* 68.68 per cent of total group of 463 cases.

this series, 197 cases (62 per cent) occurred in the decade of 16 to 25 years of age. This may be over-weighted to some extent by the student cases which were included, but the figures are in general agreement with those of others and the age distribution must be considered significant.

In Table II, the distribution of both sex and age makes evident the somewhat earlier concentration in females. Cumulative percentages show that 38.8 per cent of the female cases occurred before age 21 as compared with 25.9 per cent in males before that age. The cumulative percentage for males is 60.3 before age 26; for females, 73.8. The earlier occurrence in females is believed to be significant and will receive further attention in the discussion of the pathogenesis.

Histopathologic Features. Microscopic examination revealed that the sinus opening on the cutaneous surface usually continued into a

deeper portion which, in many instances, showed cystic dilatation (Fig. 1). Often there was evidence of branching sinuses. A stratified squamous epithelial lining, of variable degrees of integrity, was found in 235 cases (51 per cent). Of the 463 cases, 334 (72 per cent) showed

TABLE II
Incidence in Relation to Sex and Age

Age groups	Males			Females		
	No. of cases	Per cent of total	Cumulative percentage	No. of cases	Per cent of total	Cumulative percentage
0-5	2	1.0	1.0	1	1.0	1.0
6-10	0	0.0	1.0	0	0.0	1.0
11-15	2	1.0	2.0	2	1.9	2.9
16-20	50	23.9	25.9	37	35.9	38.8
21-25	72	34.4	60.3	36	35.0	73.8
26-30	30	14.3	74.6	14	13.5	87.3
31-35	24	11.5	86.1	8	7.8	95.1
36-40	13	6.2	92.3	3	2.9	98.0
41-45	7	3.3	95.6	0	0.0	98.0
46-50	2	1.0	96.6	0	0.0	98.0
51-55	2	1.0	97.6	1	1.0	99.0
56-60	3	1.4	99.0	0	0.0	99.0
61-65	1	0.5	99.5	0	0.0	99.0
66-70	1	0.5	100.0	1	1.0	100.0

Total number of known age and sex: 312.

Total number of males of known age: 209 (66.99 per cent of total).

Total number of females of known age: 103 (33.01 per cent of total).

hair shafts, either lying loose in the sinus, embedded in granulation tissue, or persisting deep in relatively mature scar tissue. In all instances, the hair stained as does "dead" hair.

A careful search was made for hair follicles which could be assigned to the wall of the sinus or cyst. In every instance in which follicles were present in the microscopic sections, it was evident that they belonged to the covering skin. The same was true of sebaceous glands, sweat glands, and arrectores pilorum muscles.

Infection and inflammation had a part in producing the general pathologic picture. Where there was no evidence of a squamous epithelial lining, the sinus was found to be lined by vascular pyogenic granulation tissue (Fig. 4). Cellular infiltrations consisted of polymorphonuclear leukocytes, lymphocytes, and plasma cells in varying proportions. Foreign body giant cells in association with dead hairs were a frequent finding (Fig. 2). In many cases there were large

mononuclear phagocytes containing blood pigment. Abscess formation deep in the tissues was present in 28.7 per cent of the cases.

DISCUSSION

From the review of the literature it appears that most writers have assumed that a pilonidal sinus results from a developmental lesion of some kind, and that the disturbance of growth has taken place at an early stage of fetal life. Among the reasons given for these assumptions are: (1) the presence in early fetal life of a connection of the skin with the neural canal; (2) the occasional presence of a deep sinus extending into the vertebral canal; (3) the occurrence of sacrococcygeal dimples; (4) the midline site of most sinuses, and (5) the presence of hair in the sinuses.

That a lesion such as a pilonidal sinus might well be associated with a deviation in anatomical structure is an obvious consideration. What is less obvious is that such a change, of which the results often are apparent only in adult life, must necessarily have occurred in the embryonic or fetal period. Hence it becomes necessary to examine more closely the arguments for the congenital origin of pilonidal sinuses.

1. Any anatomical structure or aggregation of tissues is significant only for a particular stage of development. The occurrence of a neurocutaneous communication in fetal life can be correlated intimately with skin lesions in postnatal life only by assuming that during all the changes which take place in the region of the neurenteric canal a part of this tract is relatively unaffected. Study of the stages of growth shows how completely earlier structures are changed into, or replaced by, entirely different ones. Moreover, the usual pilonidal sinus does not have tissue like that of the neurenteric canal nor does it approach the vertebral canal. There is little resemblance between spina bifida and pilonidal sinus. The former is present at birth. They occupy different sites, and the tissues involved are different.

2. Sinuses communicating with the spinal canal have been observed in the cervical and dorsal regions as well as in the lower portion of the vertebral column. Yet lesions comparable to the sacrococcygeal pilonidal sinus are not found in the superior regions.

3. Sacrococcygeal dimples are found in some infants, but no relationship to pilonidal sinuses has been observed. On the other hand, dimples are seldom found with sinuses³⁸ and at no stage of their development do pilonidal sinuses show morphologic similarities to dimples. A dimple with its smooth sloping edges and shallow base is quite different from the "pit" of a sinus, and it is always present at birth. The

sinus arises in tissues previously normal during childhood. However, there is no reason why a sinus might not develop within the area of a dimple, but evidence of a direct etiologic association is lacking.

4. The midline location of most sinuses has appeared to support the view of congenital origin on the ground of incomplete midline fusion of the lateral halves of the body. However, not all pilonidal sinuses occur in the midline^{1,2,48}; those which do not cannot be explained by this hypothesis.

5. The presence of hair in pilonidal sinuses and cyst is characteristic and is the particular feature which justifies the term in diagnosis. It was the presence of hair which attracted attention to the condition originally⁶ and which was responsible for the terminology applied to it.⁹ It is surprising to find that almost all writers subsequent to Anderson, Hodges, and Warren have assumed that the hairs arise locally, disregarding a possible extraneous origin. That the hairs do not arise from the sinus becomes quite clear when they are removed and examined. Hodges⁹ remarked: "The hairs . . . are always short, without bulbs." Sometimes a root is found, but then the root-end may be found to protrude from the mouth of the sinus. Warren,⁸ in 1867, even described the hairs as being "inverted."

Microscopically, hairs embedded deep in the sinuses are found to lie free in granulation tissue or scar tissue. They may be surrounded by foreign body giant cells. Hair follicles are never found in the walls of sinuses. These facts indicate that the hairs cannot come from within the sinuses, but are introduced from without. Such hairs might be broken off or shed locally, or they might be hairs from the back which had been shed and had then passed down between the buttocks.

The recognition of interdigital pilonidal sinuses as an occupational disease in barbers and the report of occasional examples since 1946 is obviously of prime importance in understanding the pathogenesis of the similar sacrococcygeal lesions.

In view of the objective evidence and other considerations as outlined, it seems that the theory of Patey and Scarff,² which explains pilonidal sinuses on an acquired, non-developmental basis, is better by far. In outlining a possible explanation for the occurrence of the lesions in both the postanal region and in the webs of the fingers, they divided its development into two phases: (1) an initial phase in which organisms are introduced into the tissues, there giving rise to the infection leading to sinus formation; and (2) the entrance of hairs into the sinus to produce the foreign body granulomatous reaction. Subsequent epithelization of the sinus from the surface may or may not take place, as is true, also, for the secondary abscesses and sinuses

which may form. From experiments on the possible methods of the introduction of hair into a narrow orifice, Patey and Scarff² came to the conclusion that the hairs in pilonidal sinuses are sucked in by negative pressure and are not pushed in. They believed that in the webs of the fingers and near the postanal cleft, negative pressures are readily produced; in the webs of the fingers by the alternating tautening and loosening of the tissues during movements of the fingers, and near the postanal cleft by the alternating pressure and relaxation of the soft tissues against the coccyx and sacrum during sitting. This mechanical factor has been recognized by army physicians who have called pilonidal sinuses "jeep disease."⁴⁴ The absence of negative pressures in other areas, even a short distance away from the postanal cleft, may explain why anal and ischiorectal abscesses show no tendency to become pilonidal.

The closeness of the buttocks, constant friction of skin and clothing, the accumulation of desquamated epithelium, hairs, and sebaceous material, and possible carelessness in personal hygiene make it easy to understand why the internatal region may be peculiarly vulnerable to the formation of crypts as a result of skin inflammation. Once formed, the negative pressure of the area may literally "aspirate" hairs into the pit.

Notwithstanding the experimental investigations by Patey and Scarff,² the question may well be raised whether an inflammatory sinus or pit is necessary for the introduction of the first hair. Pilonidal sinuses develop where the skin is continuously moist and where there is no horny layer. It seems possible that a short, stiff hair might be forced through the macerated epidermis mechanically, to be followed by infection, inflammation, an actual sinus, and the eventual drawing in of enough hairs to constitute a small tuft. The use of dry paper in personal cleansing after defecation may be one method by which hairs can be forced through the epidermis. Dr. K. C. Samuel⁴⁰ of the Department of Pathology of Sawai Man Singh Medical College, Jaipur, India, has stated that in his surgical material sacrococcygeal pilonidal sinuses are very uncommon. Personal cleansing after defecation is by ablution and toilet paper is never used by the native population. While this suggestion is only hypothetical, there must be some explanation for the geographic difference in incidence. Dr. Samuel is certain that the reason is not to be found in any racial dissimilarity in hairiness.

That females reach puberty earlier than males and that the sex hormones play a large part in the regulation of the growth of body hair and secondary skin structures may be concerned with the earlier ap-

pearance of pilonidal sinuses in females. With the onset of puberty there may be a relatively greater concern over personal cleanliness and greater mechanical friction from clothing in women than in men.

Hirsutism is much more common in males. Males also lead more active lives, in occupation, sports, and military service. They are less concerned with bodily cleanliness and, in general, may be assumed to have a greater accumulation of shed hairs and other detritus in the postanal region. These seem to be important reasons for the greater incidence of pilonidal sinuses in males.

It is less easy to explain the concentration of pilonidal sinuses in the period between the ages of 16 and 35. It seems not entirely adequate to note that these are the years of the rapid development of hair along with increased sebaceous gland activity, and also the years of increased physical activity. It may well be that those who, by personal habits and by character of hair and of epidermis, are proper subjects for acquiring a pilonidal sinus will have done so by age 35.

SUMMARY AND CONCLUSIONS

Study of a series of 463 cases of pilonidal sinus established that, as compared to other surgical specimens, the incidence was roughly 1:500 in the material examined. The lesion is more common in males than in females and usually becomes apparent during the second or third decade. The frequency distribution as to age and sex shows that pilonidal sinuses tend to appear about 5 years earlier in females than in males.

The pathologic picture is that of an acute or chronic inflammatory dermal sinus which contains dead hairs in about three fourths of the cases. Hair follicles are never found in the walls of these sinuses.

A review of the literature and correlation of the objective information with the various theories of pathogenesis has led to the rejection of the commonly accepted developmental or congenital theory in favor of the opinion that sacrococcygeal pilonidal sinuses and abscesses are acquired. The recognition of similar pilonidal sinuses of the interdigital web as an occupational disease of barbers provides strong evidence for a similar mode of origin for the more common sacrococcygeal lesions.

ADDENDUM

The careful study of the interdigital sinuses of barbers' hands by Currie, Gibson, and Goodall⁶⁰ was noted after this paper had been accepted. These authors reported 11 new cases of this occupational disease, bringing the total described in the literature to 29. They emphasized the importance of short, sharp hairs in causation and of personal hygiene in prevention. They prefer to restrict "pilonidal sinuses" to the postanal or perineal lesions and to designate the condition described by them as

"interdigital sinuses of barbers' hands." In the same journal, Hueston⁵¹ discussed the pathogenesis of pilonidal sinuses of the sacrococcygeal region and concluded that they are acquired lesions of foreign-body type due to the penetration of short stiff hairs.

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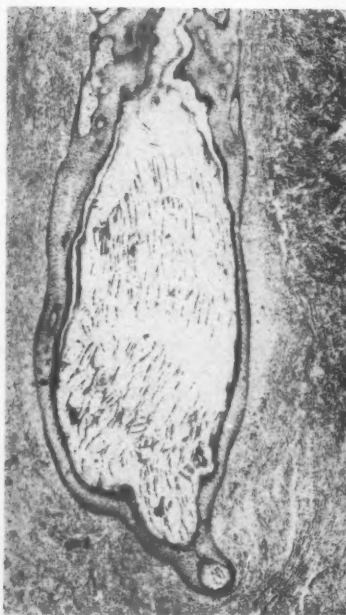
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LEGENDS FOR FIGURES

- FIG. 1. The dilated fundic portion of this epithelium-lined tract is nearly filled with dead hairs. The epithelium is intact. No hair follicles or other accessory epidermal structures are present. Hemalum and eosin stain. $\times 33$.
- FIG. 2. About twenty-five dead hairs lie nearly parallel in this pilonidal sinus. The epithelium produces abundant keratohyalin which is desquamating into the lumen. There are no hair follicles. Hemalum and eosin stain. $\times 110$.
- FIG. 3. Early foreign body giant cell reaction around dead hair shafts in the granulation tissue lining a pilonidal sinus. Hemalum and eosin stain. $\times 110$.
- FIG. 4. Dead hair shafts surrounded by newly formed connective tissue in the lumen and wall of a pilonidal sinus. Hemalum and eosin stain. $\times 110$.

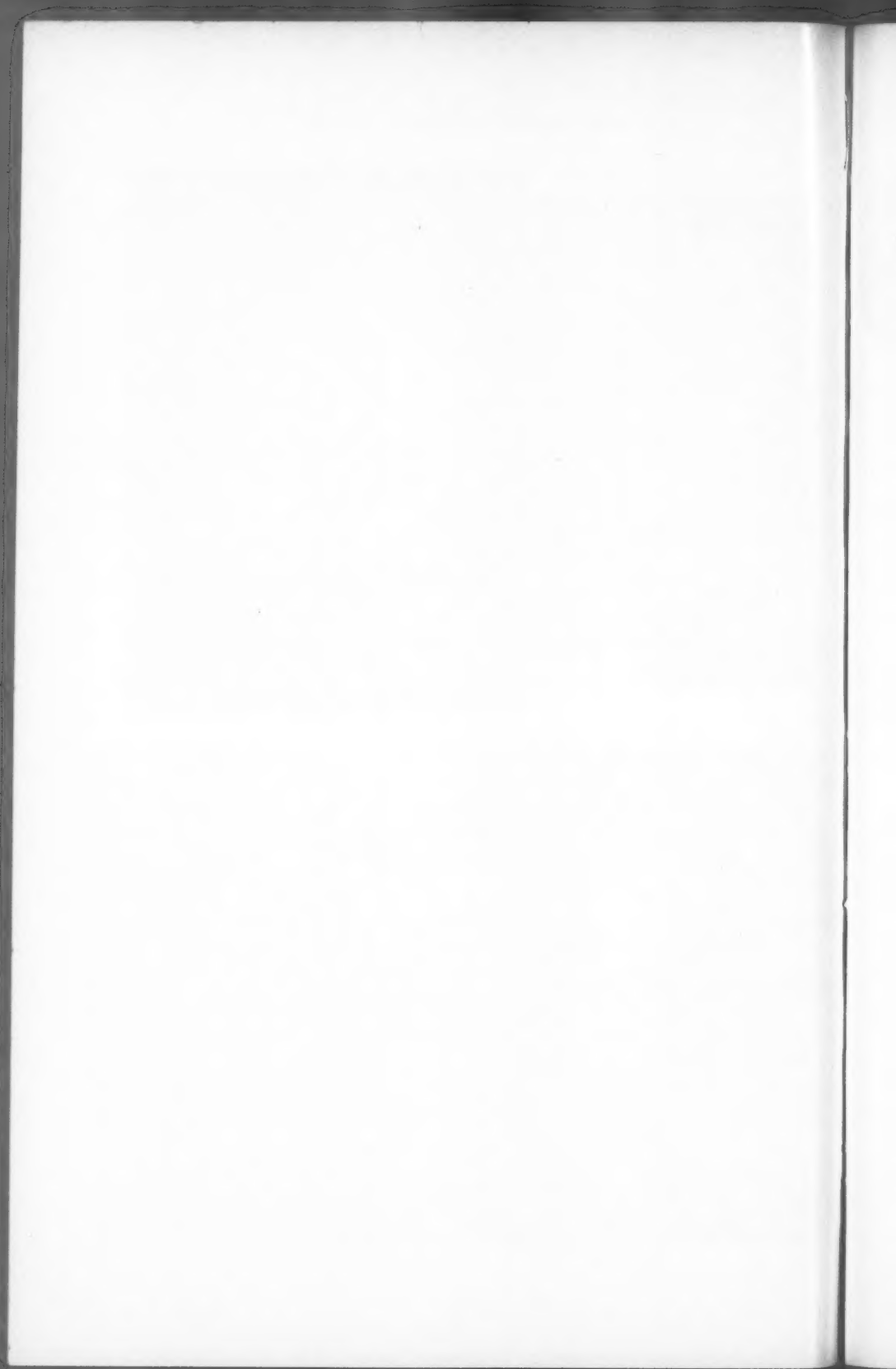
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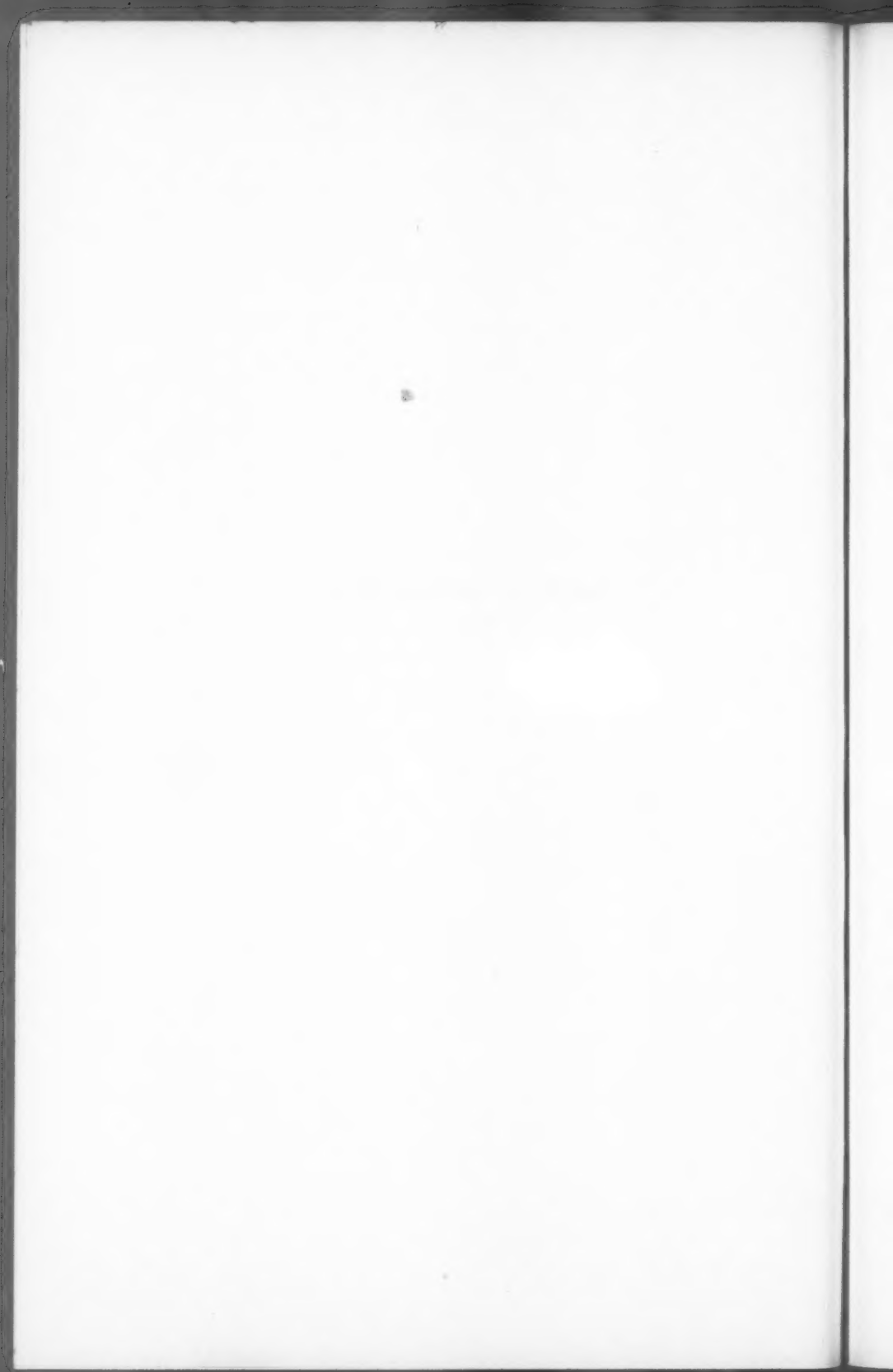


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